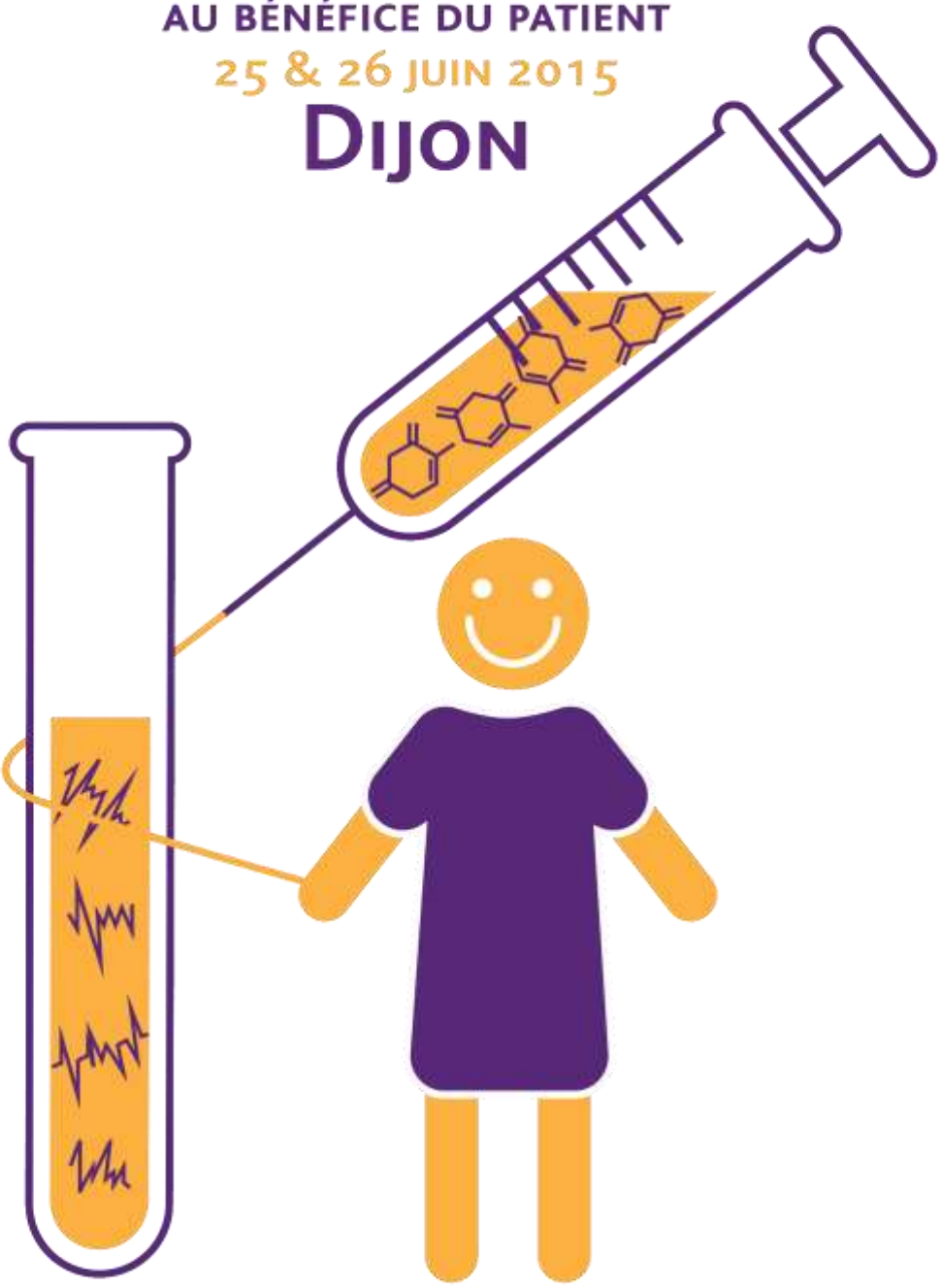
ONCOLOGIE TRANSLATIONNELLE

DU CHERCHEUR AU SOIGNANT OU DU SOIGNANT AU CHERCHEUR AU BÉNÉFICE DU PATIENT

25 & 26 JUIN 2015

DIJON



École Pratique des Hautes Études

ONCOTRANS 2015

CGFL CENTRE GEORGES FRANÇOIS LECLERC
Ensemble, dépassons le cancer

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MOT DE BIENVENUE

C'est avec plaisir que nous vous accueillons à DIJON pour ONCOTRANS 2015 !

Cette 4ème édition du Colloque Inter-régional Grand Est de Recherche Translationnelle en Oncologie est co-organisée par le CGFL, l'EPHE, l'INSERM, l'Université de Bourgogne et le CHU de Dijon sous l'égide du Cancéropôle Grand-Est.

La recherche translationnelle nécessite un travail multidisciplinaire afin de faire le lien entre les compétences des chercheurs fondamentaux et l'expertise des cliniciens-chercheurs auprès des patients. Ainsi, un lien fort entre les structures académiques de recherche, les établissements hospitaliers et les industriels impliqués en recherche clinique, notamment dans les essais précoces de phase I et II est incontournable.

A l'heure actuelle la notion de « médecine personnalisée / de précision » est au cœur du développement des thérapeutiques innovantes. Il est donc indispensable de mettre en évidence des biomarqueurs spécifiques prédictifs de l'efficacité ou de la tolérance aux nouvelles thérapies (thérapies ciblées, immunothérapie, radiothérapie …). Ces biomarqueurs compagnons sont essentiels aux cliniciens pour délivrer à leurs patients le traitement le plus adapté.

Nous souhaitons que ONCOTRANS 2015 renforce les interactions entre les différents acteurs de l'Inter-Région Grand Est impliqués dans ce domaine passionnant de la recherche au service des patients et d'accroitre la visibilité de notre réseau.

Excellent Colloque à toutes et à tous et bon séjour à Dijon !

Le Comité d'Organisation

REMERCIEMENTS

Nous avons pu réaliser le 4ème colloque ONCOTRANS grâce au soutien

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Et nous remercions particulièrement

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PROGRAMME

JEUDI 25 JUIN 2015

08h30 - 09h30 **Accueil des participants, installation des posters**

09h30 - 10h00 **Allocutions de bienvenue, ouverture du colloque -** *Pr Pierre Fumoleau (CGFL, Dijon) & Pr Pierre Oudet (Cancéropôle Grand-Est, Strasbourg)*

10h00 - 11h15 **IMAGERIE PRE-CLINIQUE ET PHASE 0: les nouveaux modèles de développement des thérapies ciblées.**

Modérateurs : *Pr François Brunotte (CGFL, Dijon) & Dr Franck Denat (CNRS, Dijon)*

- Tep-IRM : évaluation de la réponse thérapeutique des gliomes, ***Dr Samuel Valable*** *(Cyceron, Caen)*
- Imagerie optique : potentiel pour la biologie, l'innovation thérapeutique et la translation vers la clinique, ***Dr Alain Le Pape*** *(CNRS, Orléans)*
- Plateforme d'imagerie préclinique multimodale en oncologie, comment l'organiser? ***Dr Bertrand Collin*** *(CGFL, Dijon)*

11h15 - 12h00 **SYMPOSIUM LA LIGUE CONTRE LE CANCER : SOUTIEN A LA RECHERCHE**

- Implication des Comités Départementaux du Grand-Est, ***Pr Jean-François Bosset*** *(Président Comité Doubs-Besançon)*
- Fonctionnement du Conseil Scientifique Inter-Régional du Grand-Est, ***Pr Christiane Mougin*** *(Présidente du Conseil Scientifique Inter-Régional Grand-Est)*

12h00 - 13h30 **DEJEUNER, STANDS ET POSTERS**

13h30 - 16h00 **RADIOTHERAPIE/RADIOBIOLOGIE:** Les techniques de radiothérapie sont en constante (r)évolution ! De façon complémentaire, les nouvelles connaissances en radiobiologie permettent d'améliorer la prise en charge des patients en radiothérapie à la fois en termes d'efficacité et de tolérance.

Modérateurs : *Pr Philippe Maingon (CGFL, Dijon) & Pr Eric Deutsch (GR, Villejuif)*

- Impact de l'autophagie dans la réponse tumorale après irradiation, ***Pr Eric Deustch*** *(GR, Villejuif)*
- Radiosensibiliser les tumeurs en modulant leur système de réparation : exemple des i PARP, ***Dr Janet Hall*** *(INSERM, Lyon)*
- Place de l'imagerie fonctionnelle dans l'évaluation de la radiorésistance, ***Dr Sébastien Thureau*** *(Rouen)*
- Cellules souches et radiosensibilité, ***Dr Cyrus Chargari*** **(**Val de Grâce, Paris)
-

16h00 - 16h30 **PAUSE, STANDS ET POSTERS**

16h30 - 18h00 **COMMUNICATIONS ORALES SELECTIONNEES IMAGERIE ET RADIOTHERAPIE/RADIOBIOLOGIE**

Modérateurs: *Pr Philippe Maingon (CGFL, Dijon) & Pr Eric Deutsch (GR, Villejuif)*

- Tumoral Lymphocyte immune response to preoperative radiotherapy in localy advanced rectal cancer as a prognostic factor on survival: the LYMPHOREC study, **Céline Mirjolet** *(CGFL, Dijon)*
- Dosimetric and geometric commissioning of an image-guided small animal irradiator, **Sophie Chiavassa** *(INSERM U892, Nantes)*

Modérateurs: *Pr François Brunotte (CGFL, Dijon),Dr Franck Denat (CNRS, Dijon),*

- Photoacoustic imaging of hypoxia during assessment of a new highly potent antitumor prodrug in mouse modelsof human tumors, **Florian Raes** *(CNRS, Orléans)*
- Towards the elaboration of BODIPY-gold Theranostics for medical application, **Pierre-Emmanuel Doulain** *(ICMUB, Dijon)*
- Experimental approach on the minimally invaded sentinel lymph node: Bimodal investigations coupling. Photoacoustic and Near infrared Fluorescence imaging, **Serine Moussa Badiane** *(UFR des Sciences de la Santé, St Louis, Sénégal)*

19h30 **SOIREE DU CONGRÈS**

VENDREDI 26 JUIN 2015

08h00 - 08h30 Accueil des participants

08h30 - 10h10 **BIO MARQUEURS:** "Le développement des thérapeutiques ciblées est en train de révolutionner le traitement du cancer. Afin d'aboutir à une véritable médecine personnalisée, de précision, l'utilisation de biomarqueurs associés (tests compagnons) est un enjeu crucial des années à venir."

Modérateurs : *Dr Laurent Arnould (CGFL, Dijon) &Pr Pierre Oudet (Cancéropôle Grand-Est, Strasbourg)*

- Les biomarqueurs moléculaires en oncologie, **Pr Jean-Louis Merlin** *(PharmD, PhD, Université de Lorraine, CNRS CRAN UMR 7039, ICL, Nancy)*
- **Pr Gilles Favre** *(Institut Claudius Regaud, Oncopôle Toulouse)*
- Hsp 70 circulant comme biomarqueur, **Dr Carmen Garrido** *(INSERM, CGFL, Dijon)*

10h10 - 10h40 **PAUSE, STANDS ET POSTERS**

10h40 - 11h30 **COMMUNICATIONS ORALES SELECTIONNEES BIO MARQUEURS**

Modérateur : *Dr Laurent Arnould (CGFL, Dijon) & Pr Pierre Oudet (Cancéropôle Grand-Est, Strasbourg)*

- Analysis of a 33 gene panel by NGS: back on a year of experience, **Romain Boidot** *(CGFL, Dijon)*
- Regulation of the E2F1 transcription factor by the E3-ubiquitine ligase cIAP1, **Laurence Dubrez, Valérie Glorian** *(inserm umr866, Dijon)*
- Therapeutic targeting of alpha5beta1 integrin in high grade gkioma, new predictive biomarkers of efficacy, **Guillaume Renner** *(CNRS UMR7213, Illkirch)*
- Detection of KRAS, NRAS and BRAF somatic mutations in circulating tumor DNA using two assays of Next Generation Sequencing (NGS) for patients with metastatic colorectal cancer, **Alexandre HARLE** *(ICL, Vandoeuvre les Nancy)*

11h30 - 12h15 **SYMPOSIUM SYSMEX INOSTICS - MERCK SERONO**

- L'ADN tumoral circulant aujourd'hui, **Pr Pierre Laurent-Puig** *(HEGP, Paris)*
- Technologie PCR digitale BEAMing : point de vue du biologiste, **Dr Alexandre Harlé** *(ICL, Vandoeuvre les Nancy)*
- Premiers résultats cliniques en mCRC : point de vue du clinicien, **Dr Leila Bengrine** *(CGFL, Dijon)*
- Mise en application en France : études COLOBEAM et RASANC, **Pr Jean-Louis Merlin** *(ICL, Vandieuvre les Nancy)* et **Pr Pierre Laurent-Puig** *(HEGP, Paris)*

12h15 - 13h30 **DEJEUNER, STANDS ET POSTERS**

13h30 - 15h45 **IMMUNITE TUMORALE : Amie ou Ennemie ?**

Modérateurs : *Dr Olivier Adotevi (CHU, Besançon) & Catherine Paul (EPHE, Dijon)*

- Role of mucosal immunity to controlmucosal tumors, **Pr Eric Tartour** *(Paris Descartes)*
- Mécanismes d'échappement à l'immuno-surveillance dasns le cancer du sein, **Dr Christophe Caux** *(CLB, Lyon)*
- Th 17 et cancer, **Dr Lionel APETOH** *(CGFL/INSERM, Dijon)*
- TCD4 et chimiothérapie, **Pr Christophe Borg** *(CHU, Besançon)*
- Impact de l'ectoATPase CD39 sur la réponse immunitaire anti-tumorale, **Dr Nathalie Bonnefoy** *(ICM, U1194, Montpellier)*

15h45 - 16h30 **COMMUNICATIONS ORALES SELECTIONNEES IMMUNITE TUMORALE**

Modérateurs : *Dr Olivier Adotevi (CHU, Besançon) & Catherine Paul (EPHE, Dijon)*

- Dual impact of anti-mTOR therapies on antitumor CD4 T cells immunity, **Laurent Beziaud** *(INSERM, Besançon)*
- L'axe CXCL 12/CXCR4/CXCR7 dans le cancer colique humain, **Dominique Guenot** *(EA3430, Strasbourg)*
- TiO2 nanomaterials contamination could modify normal tissue response to radiotherapy through photocatalysis, **Romain Grall** *(CEA, Fontenay aux Roses)*

16h30 - 17h00 **CLOTURE DU COLLOQUE, REMISE DES PRIX DES COMMUNICATIONS ORALES SELECTIONNEES ET DES POSTERS**

JEUDI 25 JUIN 2015

SESSION 1 :

IMAGERIE PRE-CLINIQUE ET PHASE 0

CONFERENCES PLENIERES

TEP-IRM : évaluation de la réponse thérapeutique des gliomes

Dr Samuel Valable
UMR 6301 ISTCT, CERVOxy group, GIP CYCERON, Bd H Becquerel, 14074 Caen.

Les glioblastomes (GBM), tumeurs cérébrales très agressives, représentent la majorité des gliomes chez l'adulte. Suite à un traitement de première ligne composé de radiothérapie et de chimiothérapie (témozolomide) après une chirurgie, ces tumeurs récidivent invariablement nécessitant alors un traitement de seconde ligne. Toutefois, face à la faible efficacité de ces traitements et afin de renforcer cet arsenal thérapeutique, les anti-angiogéniques ont été introduits plus récemment mais la valeur ajoutée de ces thérapies ciblées appliquées en première ou seconde ligne reste encore à définir.
Actuellement, seule l'IRM anatomique est utilisée pour caractériser l'efficacité des traitements avec cependant, une faible valeur prédictive. Les informations fonctionnelles issues des récents développements autour de l'IRM mais aussi des nouveaux radiotraceurs utilisables en TEP permettraient de disposer d'outils puissants pour prédire l'efficacité de traitements ciblant les cellules tumorales ou la vascularisation.

Les objectifs de nos travaux ont donc été de déterminer l'intérêt et la valeur prédictive des biomarqueurs d'imagerie utilisables en IRM et en TEP sur des modèles orthotopiques de GBM primaires ou en récidive et traités par chimiothérapie associée ou non à un traitement anti-angiogénique.
En termes de marqueurs d'imagerie pour l'IRM, la diffusion, la perfusion, le volume sanguin et la perméabilité vasculaire ont été étudiés en parallèle de l'anatomie tumorale. Le métabolisme et la prolifération tumorale ont été évalués par TEP FDG et TEP FLT, respectivement.

Nos résultats montrent, et ce d'une manière attendue, que les marqueurs d'IRM vasculaires sont sensibles aux effets des traitements anti-angiogéniques en première ligne ou en récidive. La TEP FLT est quant à elle sensible aux effets des traitements de chimiothérapie associés ou non aux traitements anti-angiogéniques. Cependant, parmi tous les biomarqueurs d'imagerie étudiés, la TEP FLT apparait comme étant la plus robuste pour prédire l'efficacité des traitements pour les tumeurs primaires ou récidivantes.

En conclusion, la prise en charge des patients atteints de gliome devrait bénéficier de biomarqueurs d'imagerie prédictifs, robustes et précoces d'une efficacité thérapeutique dans un ère de médecine personnalisée en permettant une adaptation rapide des traitements sur la base des examens d'imagerie.

Plateforme d'imagerie préclinique multimodale en oncologie, comment l'organiser ?

Dr Bertrand Collin (CGFL, Dijon)

De nombreuses structures souhaitent installer des plateformes d'imagerie préclinique. Afin d'éviter les écueils d'un projet insuffisamment bien mené, il importe de poser au préalable un certain nombre de questions. La première concerne le type et les espèces d'animaux qui seront étudiés car ceci conditionne les modalités d'hébergement et les spécifications des appareils d'imagerie à utiliser. Sur le plan des modalités d'imagerie, l'usage de radiations ionisantes (rayons gamma et X) représente une contrainte importante, impliquant des spécificités d'appareillages et de locaux (gestion de la radioprotection). Une autre contrainte est la présence de haut champ magnétique (Imagerie de Résonance Magnétique, IRM) avec les risques s'y rapportant (personnes porteuses de *pace-maker*, objets métalliques). De fait, une telle plateforme nécessitera donc des procédures de sécurité, des restrictions d'accès, une accréditation à la fois par la Direction Départementale de la Protection des Populations et des Personnes (DDPP) et de l'Autorité de Sureté nucléaire (ASN). Dès sa conception, la présence de personnes spécialisées dans la radioactivité (personne compétente en radioprotection et radiophysicien) et de personnes accréditées pour l'expérimentation animale est indispensable. Compte-tenu des aléas liés aux sources de financements, il est impératif d'accueillir un nombre suffisant de projets successifs ou simultanés, privés ou académiques avec leurs spécificités de gestion. Il convient d'être lucide sur les rentrées financières annuelles nécessaires à l'équilibre budgétaire de la plateforme en ne mésestimant jamais les coûts de la maintenance des instruments (10 à 15% de leur prix de vente) et l'amortissement. L'ensemble de ces contraintes rend ces plateforme sensibles à leur environnement immédiat du fait des contraintes imposées ou au contraire des synergies qui peuvent être établies. Par exemple, la proximité d'un cyclotron rend plus facile l'accès à certains radiotraceurs produits sur place et permet de mutualiser des compétences (radioprotection), l'intégration à un campus hospitalo-universitaire permet une approche translationnelle des travaux s'y déroulant. Notre expérience porte sur le développement d'une plateforme d'imagerie préclinique multimodale, localisée dans le service de médecine nucléaire du Centre de lutte contre le cancer Georges-François Leclerc (CGFL). Opérationnelle depuis 2011, elle est l'une des plateformes du Groupement d'Intérêt Économique « *Pharmimage* » dont le CGFL est membre ainsi qu'une plateforme référencée au niveau régional par l'université de Bourgogne et ses partenaires. Sa conception a pris en compte les questions listées plus haut : pour les animaux, pas d'hébergement autre que temporaire, choix des souris et rats, exceptionnellement des lapins. Pour ses équipements, elle a été financée par l'Université de Bourgogne *via* des fonds publics (collectivités territoriales, Etat, Europe) à hauteur de 1,2 millions d'euros. Ses locaux ont été financés par le CGFL pour une enveloppe de 1 million d'euros et hébergent un « *Equipex* » dénommé IMAPPI (ANR-10-EQPX-05-01, Integrated Magnetic resonance And Positron emission tomography in Preclinical Imaging - 7,3 millions d'euros dont 3,8 d'investissements en matériel). Le financement du fonctionnement courant est assuré par des projets académiques et des partenariats de type public-privé, notamment avec les grandes entreprises pharmaceutiques et les sociétés de biotechnologies (oncologie, cardiologie). En 2015, la plateforme est une structure de recherche regroupant sur 475 m² tous les éléments nécessaires à la réalisation d'études d'imagerie moléculaire depuis la chimie jusqu'à l'imagerie *in vivo* du petit animal, à l'interface avec un environnement clinique fort. Cette plateforme a été organisée en trois espaces: **1°) *In vitro*** (radiochimie et culture cellulaire) ; **2°) Animalerie conventionnelle** (hébergement de souris, rats et lapins) et **3°) Imagerie** (SPECT/CT, PET/CT, PET/IRM *via* l'*Equipex* et imagerie optique). Outre l'aspect translationnel depuis la chimie jusqu'à l'imagerie moléculaire clinique, l'installation de la plateforme dans le même bâtiment que le service de Médecine Nucléaire a facilité la présence à temps partiel de techniciens, personne compétente en radioprotection, physiciens et biologistes CGFL, démontrant l'importance de la pluridisciplinarité indispensable à son fonctionnement et donc sa pérennisation.

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SESSION 2 :

RADIOTHERAPIE/RADIOBIOLOGIE

CONFERENCES PLENIERES

Impact de l'autophagie dans la réponse tumorale après irradiation

Pr Eric Deutsch
INSERM 1030 Molecular Radiotherapy, Gustave Roussy Cancer Campus & Paris-Sud university, Villejuif France.

The role of autophagy in radiation response of tumors remains largely elusive. By using a dual pharmacological and genetic inhibitory approach, we identified a dual role of autophagy in the response of cancer cells to ionizing radiation. First, we observed that the depletion of essential autophagy-relevant gene products, such as ATG5 and Beclin 1, increased the sensitivity of human or mouse cancer cell lines to irradiation, both in vitro (where autophagy inhibition increased radiation-induced cell death and decreased clonogenic survival) and in vivo, after transplantation of the cell lines into immunodeficient mice (where autophagyinhibition potentiated the tumour growth-inhibitory effect of radiotherapy). Secondly, when tumour proficient or deficient for autophagy were implanted in immunocompetent mice, defective autophagy reduced the efficacy of radiotherapy. Indeed, radiotherapy elicited an anti-cancer immune response that was dependent on autophagy-induced ATP release from stressed or dying tumour cells and was characterized by dense lymphocyte infiltration of the tumour bed. Intratumoural injection of an ecto-ATPase inhibitor restored the immune infiltration of autophagy-deficient tumours post radiotherapy and improved the growth-inhibitory effect of ionizing irradiation. Our results reveal that beyond its cytoprotective function, autophagy confers immunogenic properties to tumours, hence amplifying the efficacy of radiotherapy in an immunocompetent context. This has far-reaching implications for the development of pharmacological radiosensitizers

Radiosensitisation of tumours by modulating DNA repair: the example of PARP inhibitors

Dr Janet Hall, Hien Luong Nguyen, Inès Ahmed, Clément Guillot and Isabelle Chemin
Centre de Recherche en Cancérologie de Lyon - UMR Inserm 1052 - CNRS 5286
151 cours Albert Thomas, 69424 Lyon Cedex 03

As over 50% of all cancer patients will receive radiotherapy at some point in their treatment there is considerable interest in the development of radiosensitisers that can replace chemotherapeutic agents without the associated dose-limiting toxicities. In this respect small molecules inhibitors of poly(ADP-ribose) polymerases (PARP) are one example of such a class of drug that are entering into clinical trials. Poly(ADP-ribosyl)ation is a ubiquitous protein modification found in mammalian cells that modulates many cellular responses, including DNA repair. PARP-1, the founding member of this family is responsible for the synthesis of the majority of poly(ADP)ribose (PAR) in eukaryotic cells. Based on structural homology with its catalytic domain, 17 PARP family members have been identified of which PARP-1, PARP-2 and PARP-3 are activated by binding to DNA strand breaks and, using NAD^+ as a substrate, catalyse the formation of long homopolymers of ADP-ribose. These polymers are involved in many cellular processes and in particular the repair of DNA strand breaks. Potent and specific inhibitors of PARP activity that compete with NAD^+ at the enzyme's activity site have been developed such as Veliparib (ABT-888), Olaparib (AZD-2281), Rucaparib (AG014699) and BMN763 that enhance radiosensitivity in many preclinical *in vitro* and *in vivo* models. Such inhibitors allow the binding of PARP proteins to DNA strand breaks but inhibit their poly(ADP-ribosyl)ating activity with the result that PARP-1 and PARP-2 are trapped at DNA damage sites. These trapped PARP-DNA complexes and persistence of unrepaired DNA strand breaks due to the inhibition of PARP activity are both contributing factors to the cytotoxicity observed. PARP inhibitors can also induce temporary vasodilation increasing perfusion of tumour blood vessels. This vascular side-effect of PARP inhibitor treatment enhances drug delivery and can modulate the oxygen concentration in the tumour microenvironment and thus impact on hypoxia-related radioresistance.

Based on the strong rational of PARP inhibition in combination with radiation therapy and the fact that radiotherapy has been shown to be a promising approach for hepatocellular carcinoma (HCC) treatment (Mornex F, *et al., Int J Radiat Oncol Biol Phys.*, 66 (2006) 1152-1158; Merle P, *et al., Int J Cancer Ther Oncol.,* 2 (2014) 0204102014), with the first national randomized phase 2B clinical trial "TACERTE" comparing radiotherapy to the standard of care in the treatment of HCC recently starting, we have carried out preliminary studies to assess the potential of this combined treatment strategy in liver cancer cell lines. 4 of the 7 HCC lines were sensitive to the PARP inhibitor ABT-888 alone given as a single short 2 hour exposure and the two lines tested showed enhanced radiosensitivity in the presence of ABT-888 (Guillot *et al., BMC Cancer*, 14 (2014) 603). As HBV infection is a major cause of HCC and HBV proteins have been shown to modify the DNA damage response, these studies have now been extended to include HCC lines expressing HBV viral proteins. Our preliminary data will be presented.

Place de l'imagerie fonctionnelle TEP dans l'évaluation de l'hypoxie tumorale

Dr Sébastien Thureau
Centre Henri Becquerel – Rouen

D'un point de vue technologique, les outils modernes de la radiothérapie (imagerie multimodalité en position de traitement, planification dosimétrique avec correction des hétérogénéités d'absorption, imagerie de contrôle du positionnement sous l'appareil de traitement, irradiation avec éventuel asservissement respiratoire ou modulation d'intensité RCMI) ont considérablement amélioré la qualité technique de l'irradiation. Ces améliorations permettent d'envisager des augmentations de doses à des volumes restreints tout en respectant les contraintes dosimétriques aux organes à risque. Le volume hypoxique peut être un de ces sous-volumes cibles.

L'hypoxie tumorale est un phénomène fréquent dans les cancers notamment ceux de la sphère ORL et les cancers bronchiques et constitue un facteur important de résistance à la radiothérapie. L'existence de zones hypoxiques au sein des tumeurs et leur lien avec la radiorésistance a été établi depuis le milieu du vingtième siècle. In vitro, la dose totale de radiothérapie doit être multipliée par 3 pour obtenir le même effet cytotoxique sur des cellules hypoxiques que celui observé sur des cellules normalement oxygénées. Une telle augmentation de dose n'est pas envisageable en clinique, mais plusieurs arguments laissent penser qu'un accroissement de dose plus modeste pourrait avoir un impact positif sur le contrôle tumoral, à condition d'être mieux ciblée sur le volume hypoxique à haut risque de récidive. Paradoxalement, si le caractère péjoratif d'une hypoxie intra-tumorale est consensuel, il n'existe pas de Gold Standard pour évaluer l'hypoxie tumorale, et plusieurs techniques ont été décrites.

La méthode de référence est basée sur l'utilisation des sondes d'Eppendorf. Mais cette technique invasive ne permet pas de refléter l'hétérogénéité tumorale et d'analyser les tumeurs profondes. L'étude par immunohistochimie de marqueurs endogènes qui sont en partie régulés par l'hypoxie peut aider à définir le niveau d'hypoxie des tumeurs. La majorité des études se sont portées sur HIF et sur les protéines issues des gènes régulés par HIF : GLUT1, CA9, LOX. Ces techniques présentent l'inconvénient de ne pas prendre en compte l'hétérogénéité tumorale et le caractère indirect de ces marqueurs qui ne sont pas exclusivement régulés par l'hypoxie. Une autre méthode classique utilise des marqueurs exogènes injectés avant la biopsie ou la chirurgie qui seront réduits en condition hypoxique comme le Pimonidazole ou le Nitromidazole et qui pourront dans un second temps être analysés par immunohistochimie.

L'imagerie fonctionnelle par TEP (Tomographie par émission de positons) ou IRM permet d'obtenir une image pré-thérapeutique de l'hypoxie. Ces images permettent d'identifier les zones tumorales hypoxiques et d'envisager des augmentations de doses ciblées à ces zones. De plus les traceurs TEP de l'hypoxie notamment le F-miso peuvent être utilisés en cours de radiothérapie. Il a par exemple été démontré la disparition de la fixation F-miso en cours de radiothérapie pour les cancers de la tête et du cou et les cancers bronchiques non à petites cellules confirmant la probable réoxygénation de la tumeur en cours de traitement. Si plusieurs revues de la littérature ont souligné l'intérêt potentiel de la TEP au F-miso pour guider des modifications de traitement en radiothérapie très peu d'études ont évalué l'apport d'un complément de dose guidé par une imagerie fonctionnelle en TEP. L'étude de phase2 RTEP5 dans les cancers bronchiques dont les résultats sont en attente a permis de tester la faisabilité d'une augmentation de dose sur le volume TEP hypoxique. Une étude de phase 3 est également en cours pour les cancers ORL.

Cellules souches et radiosensibilité

Dr Cyrus Chargari
Hôpital Val de Grâce - Paris

"Au sein de l'hétérogénéité tumorale, il existe des sous populations cellulaires identifiables par des marqueurs phénotypiques des cellules souches et capables d'auto-renouvellement. Ces cellules, très tumorigènes dans des modèles animaux, semblent avoir un rôle important dans les échecs de la radiothérapie. En effet, l'irradiation pourrait dans certains cas sélectionner ces populations clonales, qui de distinguent d'autres populations cellulaires par une plus grande resistance aux agents cytotoxiques en général et à la radiotherapie en particulier. Les mécanismes de reconnaissance et de réparation des cassures de l'ADN, les interactions entre les cellules souches tumorales et leur microenvironnement, les facteurs de survie et de contrôle du cycle cellulaire, les mécanismes de transition épithelio-mésenchymateuse et les facteurs hypoxiques font partie des nombreux acteurs de cette radiorésistance et constituent autant de cibles des stratégies pharmacologiques s'intéressant aux cellules souches tumorales. Des modifications métaboliques pourraient également contribuer à la radiorésistance des cellules souches tumorales. Plusieurs travaux montrent l'intérêt preclinique à moduler certaines voies de signalisation des cellules souches cancéreuses, dont Notch, Wnt/ Bêta caténine, la survivine, chk1/2, TGFbeta ou HIF1/2. Les rayonnements ionisants à transfert linéique d'énergie élevé semblent également particulièrement adaptés pour dépasser les mécanismes de radiorésistance des cellules souches. L'identification et le ciblage des cellules souches cancéreuses sont donc autant de perspectives potentielles pour des stratégies de radiosensibilisation pharmacologique et pour améliorer l'index thérapeutique de la radiotherapie".

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COMMUNICATIONS ORALES SELECTIONNEES :

SESSIONS IMAGERIE PRE-CLINIQUE ET RADIOTHERAPIE/RADIOBIOLOGIE

TUMORAL LYMPHOCYTE IMMUNE RESPONSE TO PREOPERATIVE RADIOTHERAPY IN LOCALLY ADVANCED RECTAL CANCER AS A PROGNOSTIC FACTOR ON SURVIVAL: THE LYMPHOREC STUDY

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Auteur présentant le résumé : Céline Mirjolet

Short course preoperative radiotherapy (sc-preopRT) and long course preoperative chemoradiotherapy (lc-preopCRT) followed by a total mesorectal excision (TME) are worldwide standards of care in locally advanced T3-4 N0 or N1 rectal adenocarcinoma.
It is now well established that the host immune system participates in the elimination of the tumor cells and that a significant tumor infiltration by T-cells (LT) such as CD8+, is associated with a better prognosis.
In terms of colorectal tumors, the infiltration of Treg FoxP3+ is also described as a prognostic factor associated with a better survival.
We aimed to investigate the impact of the immune response to preoperative RT on PFS and OS in rectal cancer which underwent a TME.
237 patients with rectal cancer who underwent a TME between 1995 and 2007 after a neo-adjuvant treatment by RT with or without CT, have been analyzed in 3 french centers: the University Hospital of Besançon and the Institut de Cancérologie de Lorraine in Nancy and the Centre Georges François Leclerc in Dijon.
The LYMPHOREC study was approved by the CCP, CCTIRS and the CNIL.
Our primary objective was to assess the influence of an immune infiltration of the tumor or tumor site (in case of complete response) by CD8+ and FoxP3+ lymphocytes after sc-preopRT or lc-preopRT with or without CT on progression-free survival (PFS) and overall survival (OS). Our secondary objectives were to assess changes in the quantities of these lymphocyte infiltrations with respect to the type of preoperative RT (RT alone vs CRT) or the dose fractionation scheme (≤2Gy/fraction vs >2Gy/fraction). These second analyses were performed with 133 patients of whom one biopsy sample was collected and thus a biopsy-based pretherapeutic lymphocyte infiltration was evaluated.
Multivariate analyses, including 5 variables (chemotherapy, T, N, M and the delay between surgery and RT) were performed to evaluate the impact of immune infiltration on PFS and OS. Kruskall-Wallis test was used to study effect of RT fractionation on tumor lymphocytes infiltration.
Results: FoxP3 lymphocytes tumor infiltration after treatment significantly correlated to PFS ($p=0.007$). Variation of CD8/FoxP3 ratio before and after RT, inside the epithelial tissue, was correlated to PFS and OS ($p=0.049$ and $p=0.024$, respectively).
Interestingly, the dose per fraction (<2Gy vs. ≥2Gy) significantly influenced the CD8/FoxP3 ratio after treatment ($p=0.027$) with a lower ratio value with hypofractionated RT.
Patients with a rectal cancer who had a significant decrease in CD8/FoxP3 ratio after preoperative radiotherapy had better survival outcomes.
CD8/FoxP3 ratio needs to be validated prospectively. The immune response to preoperative RT may guide physicians in the decision of an adjuvant treatment for patients with rectal cancer.

PHOTOACOUSTIC IMAGING OF HYPOXIA DURING ASSESSMENT OF A NEW HIGHLY POTENT ANTITUMOR PRODRUG IN MOUSE MODELS OF HUMAN TUMORS

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Auteur présentant le résumé : Florian Raes

Photoacoustic Imaging (PA) is an emerging technique that is increasingly used in the field of preclinical and clinical imaging. Considering the crucial role of hypoxia upon the chemoresistance of tumors, it becomes essential for oncopharmacology studies to precisely document the hypoxic status of tumors before then during the time course of treatments. The aim of this study was to assess the efficacy of a new antitumor prodrug (a β-Glucuronidase-Responsive Albumin-Binding Prodrug of MMAE) and to characterize the tumor hypoxic status both before starting and during the treatments. Combining bioluminescence imaging (BLI) and 3D ultrasound (US) measurements allowed confrontation between tumor proliferation and tumor volumes.
Bioluminescent MIA PaCa-2 human pancreatic ductal adenocarcinoma and MDA-MB-231 human breast adenocarcinoma were orthotopically grafted, KB human mouth epidermal carcinoma being subcutaneously grafted in nude mice. Tumor proliferation was determined by BLI using the IVIS-Lumina II system (Perkin Elmer), whereas tumor volume was monitored by 3D US using the VEVOLAZR system (FUJIFILM VisualSonics Inc.). For hypoxia assessments, tumors were investigated by PA imaging so that average values of SO2 were determined and hypoxic volumes documented. Tumor perfusion status was assessed by contrast imaging following IV injection of Vevo MicroMarker™ (FUJIFILM VisualSonics Inc.).
This study demonstrates that this prodrug is significantly effective on 3 different hypoxic models of human cancer as compared to the parental cytotoxic agent (MMAE) and the vehicle group (decrease then disappearance of tumors was observed for 2 among 3 models). Due to its dependence towards O2 and ATP, BLI signals cannot be used as a relevant proliferation biomarker at the time a tumor becomes hypoxic. This phenomenon is clearly evidenced in the vehicle group that exhibit progressive decrease of BLI while tumors volumes are growing. However BLI remains a unique modality to follow early stages of proliferation and to allow allocations of animals into experimental groups with equivalent tumors stages. PA imaging provides unique resource to assess the evolution of hypoxia during the time course of these tumors and brings strong support to invalidate the use of BLI for assessment of therapies in these tumor models. 3D US measurements allow achieving accurate and reproducible determination of volumes for both superficial and internal tumors.
Considering the crucial effect of hypoxia upon chemosensitivity towards antitumor drugs, the use of fully characterized hypoxic tumor models should allow better predictivity for translation to clinical research. PA imaging coregistrated with high resolution US measurements allows ensuring a relevant assessment of antitumor therapies on hypoxic models of human tumors.
Keywords:
Tumor hypoxia, Photoacoustic imaging, Prodrug efficacy

TOWARDS THE ELABORATION OF BODIPY-GOLD(I) THERANOSTICS FOR MEDICAL APPLICATIONS

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Auteur présentant le résumé : Pierre-Emmanuel Doulain

Since the pioneer discovery of cisplatin for biological applications by Rosenberg in the 1960's, metal complexes have become the most currently investigated and used class of compounds in cancer chemotherapy.[1] Gold-based derivatives gave very promising results as anticancer agents.[2] One challenging question is to understand their mechanism of action in order to improve the efficiency while limiting their side effects. One elegant way to manage this issue consists in attaching a fluorophore on the complexes to be able to track them in vitro. Thus, we recently developed three metal-containing BODIPY-phosphine compounds based on Ru(II), Os(II) and Au(I). This first series of complexes showed promising results: interesting IC50 in several cancer cell lines, especially for the gold(I) derivative. Additionally we succeeded in following the compounds in vitro by optical imaging.[3]
In the present study, we decided to modify the physicochemical properties of gold(I) complex for it to be suitable for in vivo studies in small animals. First, the absorption and emission wavelengths of the compound were shifted to the near infrared region (in the "therapeutic window") by extension of the conjugation of the BODIPY core. In parallel, we investigated the possibility to introduce small biovectors on the gold centre for targeting selectively cancer cells (Scheme 1). The synthesis and the photophysical studies of the different targeted systems will be discussed. The biological studies will then be presented and compared with the first described compounds.[4]
References
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EXPERIMENTAL APPROACH ON THE MINIMALLY INVADED SENTINEL LYMPH NODE: BIMODAL INVESTIGATIONS COUPLING PHOTOACOUSTIC AND NEAR INFRARED FLUORESCENCE IMAGING

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Auteur présentant le résumé : Badiane Serigne Moussa

Beyond the usual tracking of labeled colloids before biopsy and histopathologic examination, the interest in exploring the sentinel lymph node (SLN), is obtaining information on the tumor invasion's stage. We considered a dynamic lymphography with the diffusible agent indocyanine green (ICG) that is detectable with both near infrared fluorescence (NIRF) and photoacoustic imaging (PA). This exploits the anatomical resolution of echography to identify SLN and to highlight changes in the micro-biodistribution of tracer to the lymph nodes (LNs). Simultaneously, the IV injection of a highly diffusible molecular probe (MP) targeting αvβ3 and αvβ5 integrins, would allow to confirm tumor micro-invasion by submitting excised LNs to high sensitivity NIRF.
For experiments, human mammary adenocarcinoma MDA-MB-231-luc model (expressing both integrins) was injected into the forepaw of female nude rats and mice to obtain a controlled invasion of axillary LN (axLN) thanks to bioluminescence imaging (BLI). In the context of coupling PA to the BLI & NIRF, we first selected the rat model because the axLN is very favorable to both in vivo and ex vivo imaging. Considering the limits in this model due to the existing residual immune-competency and the black pigmented spots on the skin, PA & NIRF were incompatible. So, we changed the protocol to the mouse model. After forepaw injection of tumor cells (TCs), BLI was performed on SLNs longitudinally, NIRF being done on the last day of study. LNs were investigated for dynamic drainage by PA imaging following an ICG forepaw injection. To target TCs, a RGD mimetic probe coupled to dyes (ex:680/em:720nm or ex:745/em:800nm) was injected IV 24 hours prior imaging. Ex vivo NIRF of excised LNs, was used with spectral unmixing to remove auto-fluorescence. An IVIS-Lumina II (Perkin Elmer) was used for BLI and NIRF, while a VEVO LAZR (FUJIFILM VisualSonics) was used for PA.
In vivo BLI is reliable to estimate discreet invasion by cancer cells in the LN (about 2000 cells to be confirmed by histopathology). PA coupled to echography was well suited to study morphology and to explore ICG filling and emptying of axLNs. This technique allowed us to identify the SLN. For fluorochromes at 680nm, ex vivo fluorescence confirmed the presence of TCs expressing αvβ3/αvβ5 integrins.
Thanks to the anatomical resolution of echography, PA imaging following ICG injection could be a promising technique for an accurate identification of SLN and associated LNs. Targeting few TCs inside a micro-invaded SLN by MP, is not sensitive enough to provide direct in vivo nor peroperative imaging. At the time NIRF is performed on the excised specimen, high sensitivity examination associated to spectral unmixing allows such a detection. For the experimental approach in animal models, BLI is a unique resource to estimate few TCs amount in vivo (less than 500 cells in a superficial area such as axLN).
Mots clés *
Sentinel Lymph Node, Ph

DOSIMETRIC AND GEOMETRIC COMMISSIONING OF AN IMAGE-GUIDED SMALL ANIMAL IRRADIATOR

Auteurs et adresses : Sophie Chiavassa, Caroline Noblet, Vincent Potiron, Stéphane Supiot, François Paris, Grégory Delpon
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Auteur présentant le résumé : Sophie Chiavassa

Radiobiologists lead basic to clinical researches on the biological responses upon ionizing radiation using a broad range of models from cell in culture to human specimens. Between all the models, rodents allow to conduct in vivo integrated radiobiological studies with the mastery of all parameters: diseases, therapeutic story, living conditions, etc.
Up to recently, preclinical studies in radiation therapy were carried out with devices that could not mimic clinical treatments: most of them consisted of a static radiation source that delivered a broad beam without integrated imaging, tumor targeting or accurate dose calculation. A new generation of preclinical irradiators narrows the technological gap with clinical devices. They consist of an x-ray tube that delivers radiation dose at medium energy range (100-300kV) whereas in clinical practice megavoltage energy range is mostly used. These kV beams allow to scale beam penetration and penumbra to the size of the small animal target volumes (few mm). Multiple beam irradiations have been made possible. Cone beam computed tomography that provides high resolution images has been integrated to allow better specimen positioning, better tumor targeting and re-irradiation. The platform also integrates an automatic motorized couch with a better accuracy than a tenth of millimeter. However, the use of these devices requires an adapted dosimetric and geometric commissioning.
The commissioning consists of dosimetric measurements, mechanical performance tests and image quality tests. Dosimetric commissioning includes half-value layer, reference dose rate, percentage depth dose curves, profiles and output factors. Due to the low energy beam and the small field size, the choice of suitable detectors is very critical. Characterization of the system geometry and mechanical flex of the X-ray tube and detector, and evaluation of image quality have to be performed, keeping in mind that due to the small size of specimen, the demands on technical precision exceed the ones for human radiotherapy.
In addition to those measurement difficulties, an important issue is the dose calculation. Absorbed dose used to be roughly estimated based on calculations in a full scatter water phantom. These estimations are not well-adapted to small animal radiotherapy given the small specimen size compared to a full scatter water phantom. Moreover such a calculation does not take into account heterogeneities (tissue density and elemental composition) that have a large dosimetric impact at kV energy range. New dose calculation algorithms mainly based on Monte Carlo simulations have to be considered.
Small animal image-guided radiotherapy is an emerging field that brings communities of biology, radiobiology, radiotherapy and medical physics in changing radiobiological research. Three French centers have recently acquired a small animal research platform and many more are considering such equipment.

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VENDREDI 26 JUIN 2015

SESSION 3 :
BIOMARQUEURS

CONFERENCES PLENIERES

Les biomarqueurs moléculaires en oncologie

Pr Jean-Louis Merlin
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Au cours de la dernière décennie, la cancérologie a vécu une évolution considérable avec le développement de stratégie thérapeutique individualisée, basée sur l'exploitation de données de diagnostic moléculaire.
Ces données peuvent être issues à la fois des caractéristiques génétiques de la tumeur mais également des caractéristiques génétiques constitutionnelles du patient, constituant autant de biomarqueurs moléculaires dont l'exploitation doit permettre d'optimiser l'efficacité thérapeutique en assurant une meilleure maitrise de la toxicité.
L'utilisation de biomarqueurs de plus en plus nombreux constitue un challenge diagnostique et doit répondre à deux besoins essentiels. Le premier est médical : améliorer les taux de réponse aux traitements et la survie des patients en assurant un meilleur rapport efficacité/toxicité. Le second est économique et concerne la maitrise des dépenses de santé en évitant la prescription de médicaments dont on peut prédire l'absence d'efficacité. C'est dans ce contexte qu'ont été créées par l'Institut National du Cancer, les plateformes hospitalières régionales, garantissant l'accès de l'ensemble de la population française à ces actes diagnostiques spécialisés.
Depuis 15 ans, nous assistons au développement croissant de thérapies dont l'utilisation repose sur un diagnostic moléculaire et l'activité des plateformes doit faire face à une activité croissante et être capables d'intégrer rapidement les innovations les plus récentes pour répondre aux besoins de la communauté médicale, au service de la population. Dans ce contexte, le développement de l'utilisation des technologies à haut débit (NGS) est en plein essor, parallèlement au concept de « biopsie liquide » reposant sur l'analyse de biomarqueurs moléculaires accessibles directement à partir d'un prélèvement sanguin. L'aboutissement de ce concept permettra la mise en place d'analyses réalisables dans des délais réduits et pouvant être réitérées au cours du suivi des patients. Dans tous les cas, les biomarqueurs identifiés devront être validés et atteindre un niveau de preuve avant d'être proposés en application diagnostique.

HSP70-exosomes: des bio-markers universels pour le diagnostique précoce du cancer

Dr Carmen GARRIDO
INSERM – CGFL – Dijon

Les métastases sont responsables de la plus part des décès liés au cancer. Le processus métastatique implique la migration et dissémination des cellules de la tumeur primaire. Les techniques utilisées aujourd'hui pour le suivi des patients (scanners, mammographies, coloscopies,…) ne permettent pas une détection précoce et ne sont pas anodines pour le patient. Pendant les 10 dernières années, la détection de cellules tumorales circulantes (CTCs) basée sur l'expression de EpCAM (Epithelial Cell Adhesion Molecule), s'est imposée comme une approche prometteuse pour la détection précoce des tumeurs. Malheureusement cette approche souffre de limitations importantes: a) la technique est très chère et seuls quelques hôpitaux peuvent en bénéficier 2) CTCs sont des évènements rares: 1 CTC/ 10^9 cellules sanguines normales; 3) l'expression de EpCAM n'est pas un marqueur du pronostique.

Mon équipe propose une approche pour résoudre ses limitations.

Nous étudions les protéines de choc thermique (**HSPs** ou aussi appelées **protéines de stress**) dans le cancer depuis plus de 20 ans. Nous avons montré qu'une de ces protéines, HSP70, est fortement exprimée dans les cellules cancéreuses mais pas dans les cellules normales et que cette surexpression augmentait d'avantage après un traitement thérapeutique. Conséquence de cette surexpression, seules les cellules cancéreuses expriment HSP70 dans la membrane. HSP70 est donc potentiellement un marqueur universel du cancer et de réponse à la thérapie. Nous avons aussi montré que les cellules cancéreuses libèrent de nano-vésicules (exosomes) qui ont HSP70 dans leur membrane (appelés **HSP70-exosomes)**. Ces exosomes tumoraux constituent un signal de danger émis par les cellules cancéreuses pour leur survie. Nous avons patenté une technique pour capturer HSP70-exosomes, basée sur l'interférence « biolayer » (BLI) et l'utilisation de ligands de haute affinité pour HSP70 que nous avons développé (aptamères peptidiques). Nous avons ainsi montré:

- Qu'une seule cellule cancéreuse peut libérer des centaines de HSP70-exosomes.
- Après avoir testé une large collection des cellules cancéreuses et normales, nous concluons que la quantité de HSP70-exosomes est importante dans le surnageant de toutes les cellules tumorales analysées pendant que cette quantité est négligeable dans le surnageant des cellules normales.
- La chimiothérapie, de façon dose-dépendante, augmente la quantité de HSP70-exosomes libérés. La libération de HSP70-exosomes semble indépendante de la mort cellulaire induite par les médicaments.
- In vivo, dans des modèles de rongeurs et chez l'homme, nous montrons que nous pouvons facilement quantifier les HSP70-exosomes aussi bien dans le sang que dans les urines.

Des études cliniques prospectives avec des patients de cancer de sein, d'ovaires et de poumon sont en cours au CGFL pour valider l'utilité des HSP70-exosomes quantifiés dans les urines et/ou le sang comme marqueurs dans le suivi de patients (détection précoce des cellules cancéreuses, marqueurs de la réponse à la thérapie).

COMMUNICATIONS ORALES SÉLECTIONNÉES :

SESSION BIOMARQUEURS

ANALYSIS OF A 33 GENE PANEL BY NGS: BACK ON A YEAR OF EXPERIENCE

Auteurs et adresses : Sandy Chevrier1,4, Françoise Beltjens2, Laurent Arnould2,4, François Ghiringhelli3,4, Bruno Coudert3, Sylvie Zanetta3, Isabelle Desmoulins3, Aurélie Lagrange3, Laure Favier3,Julie Vincent3, Nicolas Isambert3, Leila Bengrine3, Véronique Lorgis3, Sylvain Ladoire3,4, Romain Boidot1,4
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Auteur présentant le résumé : Romain BOIDOT

The development of targeted therapies in cancer has accelerated the development of molecular diagnosis. This new cancer discipline is booming, with an increasing number of gene alterations to analyze in a growing number of patients. To deal with this fast-developing activity, current analysis techniques (Sanger sequencing, allelic discrimination and high resolu¬tion melting) take more and more time. In recent years, next generation sequencing (NGS) technologies have appeared and given new perspectives in oncology.
In this study, we analyzed FFPE tumor samples from patients in therapeutic failure who were treated at the Centre Georges-François Leclerc. We developed a TruSeq Custom Amplicon (Illumina) panel for the analysis of 100 exons spread over 33 genes. The amplicon length is based on 250 bp. From June 2014 to March 2015, we succeed in library preparation in 92% (119/129) of samples.
We observed 6 non-synonymous single nucleotide polymorphisms (SNP) whose 2 are described as modifying the activity of the protein (Q472H for KDR gene, and V189I for SLC28A1 gene). We identified 266 non-synonymous mutations whose 235 different mutations, resulting in an average mutation rate of more than 2 (0-12) mutations per patients. When we classified the mutational rate by origin of cancer, it appeared that head and neck cancers are the most altered (> 4 mutations/patient), followed by digestive and urinary tract cancers (3 mutations/patients), lung cancers (2 mutations/patient), and gynecological cancers (<2 mutations/patients). The most mutated gene is TP53 (about 44% of tumors), followed by APC (about 31% of samples). By comparison with routine diagnosis, 50% (8/16) of EGFR mutations, 20% (3/15) of KRAS mutations, 100% (5/5) of NRAS mutations, 60% (3/5) of BRAF mutations, and 27% (3/11) of PIK3CA mutations observed with our NGS design are not routinely searched. Moreover, we observed 3 ERBB2 gene mutations in cancer samples (2 head and neck and 1 colon) for which ERBB2 alterations are not routinely studied. We also observed 7 HRAS mutations that could be linked to resistance to some targeted therapies. As the number of alterations detected is important, the use of results by clinicians is sometimes difficult. As in Centre Georges-François Leclerc, the use of NGS is for patient care, a molecular meeting with clinicians and a molecular biologist occurs once a month. Therapeutic recommendations are proposed and the clinician chooses to follow or not the proposals.
In conclusion, despite the important number of unknown mutations (about 40% of mutations), 30 to 40% of analyzed patients harbored a mutation profile that could be helpful for therapeutic decision. The use of NGS analysis with an enlarge panel of genes is interesting for patients with a metastatic disease. When more targeted therapies will be available in France, this analysis tool will be an advantage for clinicians in order to use the best treatments for their patients.

REGULATION OF THE E2F1 TRANSCRIPTION FACTOR BY THE E3-UBIQUITINE LIGASE CIAP1

Auteurs et adresses : Glorian Valérie, Boutanquoi Pierre-Marie, Berthelet Jean, Allègre Jennifer, Dubrez Laurence
IAP team, Centre de Recherche Lipides, Nutrition, Cancer, UMR866 Inserm /Université de Bourgogne-Franche-Comté, Dijon, France

Auteur présentant le résumé : Valérie GLORIAN et Laurence DUBREZ

The cellular inhibitor of apoptosis 1 (cIAP1) is a member of IAP family with oncogenic properties. It is a potent regulator of cell signalling controlling several fundamental biological processes including innate immunity, inflammation, cell death, cell migration and cell proliferation. The importance of these different cellular functions for the oncogenic activity of cIAP1 is not well understood. In order to identify the molecular mechanism responsible for the proliferative activity of cIAP1, we engaged the analysis of the interactome and identified the transcription factor E2F1 as a novel partner of cIAP1. E2F1 is a fundamental regulator of the cell cycle progression. We demonstrated that cIAP1 can stimulate E2F1 activity and cell proliferation (Cartier & Berthelet et al. J. Biol. Chem. 2011). The present work aimed to identify the mechanisms of regulation of E2F1 by cIAP1. cIAP1 is endowed with an E3-ubiquitine ligase activity, able to conjugate ubiquitin or nedd-8 chains to protein partners. We showed that the E3-ubiquitine ligase activity of cIAP1 is required for the regulation of E2F1. cIAP1 is able to induce a non degradative ubiquitination of E2F1. While acetylation of E2F1 did not interfere with cIAP1-mediated E2F1 regulation, an inhibition of the Protein Arginine Methyltransferases (PRMTs) using AMI-1 prevents the regulation and ubiquitinilation of E2F1 by cIAP1.
cIAP1 overexpression induces a stabilisation of E2F1 protein expression and silencing of cIAP1 prevents the etoposide-induced E2F1 stabilisation, suggesting that cIAP1 could regulate E2F1 in a DNA damage context.

THERAPEUTIC TARGETING OF ALPHA5BETA1 INTEGRIN IN HIGH GRADE GLIOMA; NEW PREDICTIVE BIOMARKERS OF EFFICACY.

Auteurs et adresses : RENNER Guillaume1, JANOUSKOVA Hana1, NOULET Fanny1, Eric GUERIN2, Severine BÄR3,4, Jürg NUESCH3, Horst KESSLER5, and Monique DONTENWILL1*.
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Auteur présentant le résumé : Guillaume RENNER

Integrins are implicated in several, if not all, hallmarks of cancer. These αβ heterodimeric transmembrane proteins sense the cell microenvironment and thus activate intracellular signaling pathways. They are major players in tumour cell survival/resistance to apoptosis, migration/invasion and therapy resistance. However, targeted therapies with integrin antagonists gave disappointing results. Basic knowledge on integrin activities in cancer cells are still not enough understood to overcome therapeutic difficulties.
We previously demonstrated that α5β1 integrin, the fibronectin receptor, represents a therapeutic target for high grade glioma as its overexpression is associated with a bad prognosis. We also showed that α5β1 integrin negatively impacts on chemotherapy-induced p53 activation leading to Temozolomide (glioblastoma standard of care) resistance. Here, we characterized the molecular pathways underlying the negative crosstalk between α5β1 integrin and p53 in glioma cell lines to select for new biomarkers of integrin inhibition efficacy.
The restoration of p53 tumor suppressor function is under intensive investigations for cancer therapy. Although direct p53 activation may be achieved by molecules such as Nutlin-3a, apoptotic cell outcome is not always achieved. We investigated whether integrin α5β1 functional inhibition or repression could sensitize glioma cells to Nutlin-3a-induced p53-dependent apoptosis. We discovered that α5β1 integrin specific blocking antibodies or small RGD-like antagonists in association with Nutlin-3a triggered a caspase8/caspase3-dependent strong apoptosis in glioma cells expressing a functional p53. We showed that two anti-apoptotic proteins, PEA-15 and surviving, are crucial in glioma cells apoptotic outcome. PEA-15 is under α5β1 integrin/AKT control and survivin is a p53-repressed target. These two proteins delineate the interconnected pathways between integrin α5β1 and p53. Indeed, PEA-15 repression by specific siRNA activated p53 pathway to repress survivin and conversely survivin repression by specific siRNA decreased α5β1 integrin expression. This pro-apoptotic loop identifies a novel mechanism depending both on α5β1 integrin expression and p53 functional status in glioma. Interestingly, association of integrin antagonists with repression of survivin recapitulates the apoptotic outcome in p53 mutant cells.
Our data suggest that integrin targeted therapies must be addressed to a selected population of patients and that pertinent therapeutic drug associations must be used. To include α5β1 integrin antagonists in glioma radio/chemotherapeutic treatment, clinically relevant biomarkers may at least include a high level of α5β1 integrin in tumor as well as a functional p53 protein.
Alternatively, integrin antagonists associated with survivin repressors may be useful in glioma whatever their p53 status.

DETECTION OF KRAS, NRAS AND BRAF SOMATIC MUTATIONS IN CIRCULATING TUMOR DNA USING TWO ASSAYS OF NEXT GENERATION SEQUENCING (NGS) FOR PATIENTS WITH METASTATIC COLORECTAL CANCER.

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Background: Colorectal cancer (CRC) is one on the most diagnosed cancer with more than one million new cases every year in the world. Presence of somatic mutation on exons 2, 3 and 4 of KRAS or NRAS genes is a validated anti-EGFR (i.e. cetuximab or panitumumab) resistance biomarker in patients with metastatic CRC (mCRC). Mutations on exon 15 of BRAF gene is a validated biomarker of poor prognosis in patients with mCRC. DNA extracted from formalin-fixed paraffin embedded (FFPE) tumor fragments is commonly used for KRAS, NRAS and BRAF genotyping. Unfortunately, FFPE fragments are not always available and sometimes tissues are not suitable for DNA extraction (i.e. irradiation, necrosis or DNA over fragmentation, bone metastasis). It is now admitted that DNA released from tumor cells can be found in blood, and is identified as circulating tumor DNA (ctDNA). In this study, we used next generation sequencing (NGS) for the detection of ctDNA in plasma and the identification of somatic mutations on KRAS, NRAS and BRAF.
Methods: Blood samples from mCRC patients with known KRAS, NRAS or BRAF tumor mutations were collected using 10mL cell free DNA BCT blood collection tubes (Streck) and centrifuged for 5 min, 2000 rpm at room temperature. Supernatants were centrifuged a second time at 12000g, +4°C during 15 min to pellet residual contaminating cells. Plasmas were stored at -80°C until DNA extraction. ctDNA was extracted from plasma using QIAamp circulating nucleic acid kit (Qiagen). Libraries were prepared by two different methods, using homemade primer design or Truseq custom amplicon kit (Illumina). NGS was then performed using ultra deep pyrosequencing (GS Junior, Roche Diagnostics,) or bridge PCR sequencing (MiSeq, Illumina).).
Results: Both NGS assays allowed the detection of somatic mutations of KRAS, NRAS and BRAF in plasma. A good concordance between mutations found in plasma and in tumors was observed. Further investigations in a larger series of samples are ongoing to confirm these preliminary data.
Conclusion: NGS is suitable for the detection and characterization of somatic mutations in ctDNA in plasma. These data suggest that liquid biopsy could replace standard biopsy for patients with no available FFPE tumor fragments or for patients who cannot have an invasive tissue biopsy. Moreover, NGS will allow the analysis of large gene panels from a 10 mL blood sample.
This study was performed with the financial support of Cancéropole Grand Est.

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SESSION 4 :

IMMUNITE TUMORALE

CONFÉRENCES PLÉNIÈRES

Rôle de l'immunité anti-tumorale muqueuse dans le contrôle des tumeurs muqueuses de la sphère ORL et pulmonaire (Rôle of Mucosal immunity to control mucosal tumors)

Pr Eric Tartour.
INSERM U970. Hôpital Européen Georges Pompidou. Université Paris Descartes.

De nombreuses tumeurs sont de localisation muqueuse (poumon, colon…). Nous avons montré dans un modèle de tumeur orthotopique de la sphère ORL exprimant les antigènes E6 et E7 d'HPV 16 qu'un vaccin thérapeutique anti-HPV administré par voie nasale induisait des lymphocytes T (LT)-CD8 anti-E7 de localisation muqueuse (ORL et pulmonaire) et entrainait une regression tumorale. Au contraire le même vaccin administré par voie intramusculaire n'induisait que des LT-CD8 anti-E7 dans le sang et n'était pas efficace dans la protection contre des tumeurs muqueuses (Sandoval et al Sci Transl Med 2013).
Nous avons ensuite caractérisé ces LT-CD8 spécifiques d'antigènes et montré que les LT-CD8 muqueux exprimaient des marqueurs (CD49a, CD103) définissant les LT-CD8 résidents muqueux (Trm). Ces cellules sont induites après vaccination muqueuse et persistent dans les muqueuses même après l'élimination de la tumeur. Les cellules dendritiques muqueuses pulmonaires permettaient d'induire ce phénotype de Trm sur les lymphocytes T $CD8^+$ alors que les cellules dendritiques de la rate ne possédaient pas cette propriété. Afin de montrer le rôle de ces LT-CD8 muqueux dans le contrôle de la prolifération des tumeurs, nous avons réalisé une série d'expériences : i) l'élimination des LT-CD8 inhibe l'efficacité du vaccin administré par voie muqueuse. ii) l'utilisation du FTY720 (fingolimod), une molécule qui induit l'internalisation et la dégradation des récepteurs de la sphingosine 1 phosphate (S1P1…) et la rétention des lymphocytes dans le ganglion et le thymus a permis de montrer le rôle de ces LT-CD8 muqueux, car la vaccination muqueuse reste partiellement efficace dans le contrôle des tumeurs muqueuses même en présence de cet inhibiteur de migration. Ces expériences signifient que même en l'absence d'un recrutement de LT-CD8 périphériques, les Trm jouent un rôle dans la protection tumorale iii) En modulant la proportion respective de LT-CD8 systémique et résidents, nous avons montré que la baisse du ratio Trm/LT périphériques était corrélée à la perte d'efficacité du vaccin.
Pour montrer la pertinence de ces résultats chez l'homme, ces LT résidents mémoires ont été retrouvés chez des patients atteints de cancers ORL et pulmonaires.
Nos résultats ouvrent de nouvelles perspectives dans le développement de vaccins thérapeutiques contre les cancers muqueux et soulignent la nécessité d'induire ces Trm dans pour l'efficacité clinique de ces vaccins anti-cancers

Study of immuno-surveillance and immune escape in breast and ovarian carcinoma

Dr Christophe Caux
Team "Targeting of the tumor and its immune environment"
Lyon, Cancer Research Center, director Alain Puisieux

Although targeted therapies have greatly improved the prognosis of several tumors, they do not achieve complete remission and do not prevent secondary resistance. In addition, while the immune system is one of the main barriers protecting the body against tumor development, the anti-tumor immune response is compromised in cancer patients. Co-directed by JY Blay and C Caux - bringing medical and scientific expertise, our team conducts a translational research in Breast (BC) and Ovarian (OC) carcinoma aiming at the identification of immune escape mechanisms and the development of therapeutic strategies to restore anti-tumor immune responses.
The team shows that 20-25% of patients with advanced cancer have lymphopenia and impaired TCR diversity of T lymphocytes (divpenia) which impacts overall survival (**1**). This observation led us to conduct a clinical trial at CLB using IL-7 (collab Cytheris), a growth factor of T cells, to restore this impairment.
At the stage of primary localised tumor, the team contributed to show the critical role of dendritic cells (DC) in T cell activation and pioneered several escape mechanisms from immunosurveillance in BC and OC such as altered myeloid DC (mDC) physiology, selective plasmacytoid DC (pDC) functional alteration, and dominant local Treg activation.
In particular we show that the presence of pDCs in BC and OC negatively impacts tumor progression (**2**), and that the tumor environment, via TGFβ/TNFα alter the primary function of pDC to produce IFNa (**3**). The presence of regulatory T cells (Treg) in the primary tumor is also a poor prognostic factor. Treg cells are selectively recruited (via CCL22 / CCR4) and activated in the periphery of the tumor where they inhibit the function of conventional T cells (**4**). We demonstrate the major role of the interaction ICOS / ICOSL in the expansion of Treg by pDCs in the tumor (**5**). Thus, our current observations strongly suggest that pDC/Treg interactions play a pivotal role in the local immuno-suppressive network of BC and OC, and identify targets for immune intervention.
Our project is based on the hypotheses that residual tumor cells that escape targeted therapies may be eradicated through the i) the correction of immune alterations induced by tumor cells and ii) the induction of a specific anti-tumor immune response. The induction of immune memory should lower the emergence of secondary resistance and prevent relapse even after the treatment has ended. This strategy is supported by the recent results of clinical trials using immune check point antibodies (CTLA4, PD1) demonstrating the possibility to reverse immunosuppression and to obtain potent and long lasting therapeutic responses.

Références : **1** - M. Manuel, et al ***Oncoimmunol*** 2012. **2** - Labidi-Galy SI et al ***Cancer Research*** 2011; Hirsch I et al ***Trends Immunol*** 2010. **3**-V Sisirak et al ***Cancer Research*** 2012; V Sisirak et al ***Int J Cancer*** 2013. **4**- Gobert M et al ***Cancer Research*** 2009 ; Ménétrier-Caux C et al ***Cancer Research*** 2009; Faget J et al ***Cancer Research*** 2011; Dercamp C et al ***Cancer Research*** 2005. **5-** Faget J et al ***Cancer Research***, 2012.

Cellules Th17 et cancer

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Bien que le rôle proinflammatoire des cellules T CD4 productrices d'IL-17 (cellules Th17) ait été mis en évidence dans les pathologies autoimmunes, le rôle des cellules Th17 dans le cancer reste controversé. Nous avons étudié les fonctions des cellules Th17 dans différents modèles de cancers murins. Nos résultats montrent que l'expression des ectonucléotidases par les cellules Th17 détermine leurs fonctions immunosuppressives. Nous avons pu par ailleurs mettre en évidence que l'acide docosahexaénoïque (DHA), acide gras appartenant à la famille des acides gras polyinsaturés n-3, inhibe directement la différenciation des cellules T CD4 naïves en cellules Th17. La capacité du DHA à bloquer la différenciation cellulaire Th17 est dépendante du facteur de transcription PPAR· et le DHA prévient la croissance tumorale induites par les cellules Th17 *in vivo*. Enfin, nos données indiquent que les cellules Th17 sont également responsables du blocage de l'induction de réponses immunitaires antitumorales en réponse à l'administration de 5-Fluorouracile, une drogue de chimiothérapie utilisée dans le traitement du cancer colorectal. Cette présentation s'attachera à présenter différentes stratégies thérapeutiques visant à restaurer des réponses immunitaires anticancéreuses optimales en bloquant la génération de cellules Th17 *in vivo*.

Impact de l'ectoATPase CD39 sur la réponse immunitaire anti-tumorale

Dr Nathalie Bonnefoy
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CD39 est une ectonucleotidase exprimée par différentes cellules immunitaires notamment les lymphocytes T régulateurs ainsi que certaines cellules tumorales. CD39 hydrolyse l'ATP extracellulaire et l'ADP en AMP qui est ensuite converti en adénosine par une seconde ectonucléotidase, CD73. L'adénosine est un puissant immunosuppresseur qui se fixe sur les récepteurs A2A à la surface des cellules T effectrices (TCD4, TCD8 cytotoxiques et NK) et entraîne l'accumulation d'AMPc inhibant ainsi la prolifération, la sécrétion de cytokines pro-inflammatoires et l'activité cytotoxique des cellules T effectrices et NK. Outre la génération d'adénosine, l'expression de CD39 est également responsable de la diminution de l'ATP extracellulaire, un immuno-activateur essentiel à la mise en place d'une réponse immunitaire anti-tumorale efficace en augmentant notamment la présentation des antigènes tumoraux par les cellules dendritiques matures (pour revue voir Bastid et al., Oncogene 2013).
Récemment, nous avons analysé, par immunophénotypage en cytométrie de flux, les cellules du microenvironnement tumoral dans un modèle préclinique de mélanome murin (B16F10). Nous montrons que la progression tumorale chez des souris non traitées et dans le contexte d'un échappement à un traitement par un anticorps spécifique d'un antigène tumoral (traitement ciblé), est associée à la présence de lymphocytes T effecteurs de phénotype «épuisé» (co-expression des marqueurs PD-1 et Tim3) et à un environnement fortement immunosuppresseur avec, en particulier, la présence de lymphocytes T régulateurs, mais aussi de lymphocytes T effecteurs CD4 et CD8 exprimant les ectonucléotidases CD39 et CD73 (données non publiées). Au delà des cellules immunitaires du microenvironnement, nous avons montré que CD39 était également exprimée à la surface de plusieurs types de cellules tumorales humaines dont le mélanome et que son expression est associée à l'inhibition de la prolifération des lymphocytes T et des fonctions cytotoxiques des cellules NK par les cellules tumorales elles mêmes (Bastid et al., Cancer Immunol Res 2014, Bonnefoy et al., Oncoimmunology 2015). L'ensemble de ces résultats suggère qu'une des limitations potentielles à la mise en place d'une réponse anti-tumorale protectrice efficace, est l'inhibition des effecteurs anti-tumoraux par la voie CD39/CD73/adénosine. Dans ce contexte inhiber la molécule CD39 apparaît comme une stratégie prometteuse pour limiter l'immunosuppression liée à l'activité de l'ectoenzyme CD39.

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COMMUNICATIONS ORALES SELECTIONNEES :

SESSIONS IMMUNITE TUMORALE

DUAL IMPACT OF ANTI-MTOR THERAPIES ON ANTITUMOR CD4 T CELLS IMMUNITY

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Auteur présentant le résumé : Laurent Beziaud

The mammalian Target Of Rapamycin (mTOR) pathway plays a central role in the regulation of cell growth and metabolism, and is involved in oncogenesis. Everolimus and temsirolimus are two mTOR inhibitors approved for renal and breast carcinoma treatments. However, accumulating evidence highlights a central role for mTOR pathway in CD4 helper T cell differentiation. Hence, mTOR inhibition induces regulatory CD4 T cells (Treg) expansion but can also promote a subset of high quality memory CD8 T cells responses. Here we concomitantly studied Treg and antitumor Th1 responses in metastatic renal cell carcinoma (mRCC) patients treated with everolimus and used in vivo tumor model to explore the impact of adaptive immune T cells on mTOR inhibitors efficacy.
A monitoring of Tregs in twenty-three mRCC patients was performed in blood before and during everolimus treatment. Antitumor T cell reactivity was evaluated by IFN-· ELISpot assay using telomerase as tumor antigen. The inhibitory properties of Tregs after anti-mTOR treatment of T cells from healthy donors were analyzed in vitro.
OVA-expressing murine melanoma tumor cell line (B16-OVA) was graft in vivo in T cells-depleted mice treated with either temsirolimus or everolimus. Foxp3-eGFP-DTR mice (Foxp3-DTR) were used to study the impact of Tregs during anti-mTOR treatment.
We observed that 21 out of 23 of mRCC patients had an increase of Tregs after everolimus treatment. Paradoxically, strong antitumor Th1 responses were detected in patients and these responses greatly decreased at the time of disease progression when high expansion of Treg cells occurred. In vitro, Treg cells exposure with everolimus or temsirolimus highly suppressed T cells proliferation and Th1-associated cytokines production.
In mouse tumor model, we showed that T cells subset depletion differentially modulate the efficacy of temsirolimus or everolimus treatment. Although antitumor effect was loss in B16-bearing mice lacking CD8 T cells, CD4 depletion increased everolimus or temsirolimus efficacy. Then, we showed that conditional depletion of Foxp3+ Treg cells in B16-OVA-bearing Foxp3-DTR mice greatly enhanced anti-mTOR treatment efficacy by promoting robust anti-OVA CD8 T cell responses.
These data suggest that mTOR inhibitors shape host immune antitumor T cell responses, which in turn could contribute to the efficacy of the anti-mTOR therapy. It could be interesting in the future to combine mTOR inhibitors with Treg blockade therapies such as anti-angiogenic, anti-CTLA4, and anti-CCR4.

L'AXE CXCL12/CXCR4/CXCR7 DANS LE CANCER COLIQUE HUMAIN

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Auteur présentant le résumé : Dominique Guenot

Dans les cancers, la chimiokine CXCL12 est connue pour favoriser la prolifération cellulaire et les métastases. Elle est exprimée de façon constitutive dans des sites connus pour être des organes cibles de développement de métastases et la perte d'expression dans la tumeur primaire et la forte expression aux sites de métastases suggèrent qu'il se crée un gradient attractif entre les cellules tumorales primaires n'exprimant plus CXCL12 et la niche métastatique. D'autre part, l'expression de CXCR4, un des récepteurs de CXCL12, faible ou absente dans de nombreux tissus sains, est augmentée dans les tissus tumoraux et le maintien d'un niveau élevé de CXCR4 permettrait aux cellules tumorales circulantes de s'installer dans les organes exprimant des niveaux élevés de CXCL12, et avec CXCR7, favoriserait l'extravasation endothéliale des cellules tumorales et le développement de métastases. Objectif : Evaluer les niveaux d'expression et mieux comprendre les mécanismes de régulation de l'expression de CXCL12 et des récepteurs CXCR4 et CXCR7 et leur lien à l'hypoxie et au facteur HIF-1 dans la dissémination du cancer colique. Résultats : Nos données indiquent une perte d'expression du transcrit codant CXCL12 dans la majorité des adénomes et des carcinomes colique humains, et l'intensité de la perte n'est pas corrélée au stade tumoral. De même, CXCL12 n'est plus exprimée dans des tumeurs qui se développent chez des souris traitées par un carcinogène (azoxyméthane), ou chez des souris mutées pour le gène APC (Souris APC Min et APC Δ14). Des analyses du méthylome indiquent que seules 30% des tumeurs ont un promoteur méthylé mais l'inhibition des HDAC par du butyrate ou du valproate augmente le niveau d'acétylation des histones H3 du promoteur de CXCL12 et restaure l'expression de la chimiokine dans des lignées coliques. Sur le plan fonctionnel, le butyrate et le valproate inhibent significativement la migration cellulaire (> 90%) induite par CXCL12. D'autre part, l'hypoxie induit fortement l'expression de CXCR4 à la membrane cellulaire, expression régulée par HIF-1α, tandis que l'expression de CXCR7 est indépendante de l'hypoxie. Après une hypoxie transitoire et le retour à la normoxie, le niveau d'expression de CXCR4 reste stable à la membrane pendant au moins 48 heures. Conclusion : La perte d'expression de CXCL12 est un événement qui cible précocément le cancer colique et peut être considérée comme un marqueur de la transformation de la muqueuse intestinale. Un défaut d'acétylation des histones expliquerait l'extinction de CXCL12. D'autre part, l'hypoxie favorise une forte expression de CXCR4 à la membrane des cellules tumorales via HIF-1a, alors que l'expression de CXCR7 n'est pas altérée. Un ciblage simultané des deux récepteurs CXCR4 et CXCR7 pourrait avoir un intérêt thérapeutique dans les cancers coliques puisque leur perte d'expression diminue la capacité migratoire de cellules tumorales.

TIO2 NANOMATERIALS CONTAMINATION COULD MODIFY NORMAL TISSUE RESPONSE TO RADIOTHERAPY THROUGH PHOTOCATALYSIS

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Auteur présentant le résumé : Romain Grall

The use of nanomaterials (NMs) has dramatically increased in the last decades and they are widely involved in many engineered products, particularly nano titanium dioxide (TiO2). Unlike bulk form of TiO2, the nanosized compound has specific physical, chemical and biological properties, related to its surface reactivity. Among them, photoactivity is one of most interesting for medical applications since it generates reactive oxygen species (ROS) in cells after photocalysis activation. By using this ROS production, promising outcomes are expected for TiO2 NMs with UV photodynamic therapies or to increase the effectiveness of X-ray irradiation in oncology. Since the photoactivation of TiO2 NMs generates ROS known to be cytotoxic and genotoxic, the hazards related to TiO2 NMs photoactivation after occupational (application of skin care products or inhalation) should be addressed. Photoactivation could occurred on skin after non ionizing UV natural sun light exposure, but also potentially on all normal tissues exposed to IR at low doses for diagnostic imaging (X ray, mammography and scanner) or at high doses in case of radiotherapy. In case of radiotherapy, the dose delivery is indeed higher in the tumor however surrounding normal tissues in the field of exposure are also exposed.
In the present work we addressed the question whether TiO2 NMs could modify the tissue response after IR exposure. We analyzed the potential alteration in radiation sensitivity of two human lung cell lines A549 and Calu-3 co-exposed to TiO2 NMs and gamma rays at doses of IR compatible with those used in radiotherapy and of TiO2 NMs compatible to what is expected in case of lung inhalation.
We show that commercial P25 TiO2 NPs alone do not induce any cytotoxicty on two human pulmonary cell lines A549 and Calu-3 in absence of sufficient photo-activation as previously shown. However, the combined exposure of TiO2 P25 with gamma rays, at doses that do not induce any toxicity, neither for radiation alone nor for TiO2 alone, increases the radiation sensitivity of the two human pulmonary cell lines A549 and Calu-3.
Occult contamination by NMs presenting a photocatalytic activity, such as TiO2, which is so widespread in usual products, may be at the origin of some cases of high radiation sensitivity. The hazards of TiO2 contamination regarding the sensitivity of healthy tissues in response to low doses of ionizing radiation used for diagnostic imaging deserve to be explored.

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POSTERS

POSTERS SELECTIONNES

THEMES : A = Autres thèmes B = Biomarqueurs
I : Imagerie Pré-Clinique IT = Immunité Tumorale
R : Radiothérapie/radiobiologie

1er auteur	Thème	N° Poster	Titre
STALIVIERI Aurélie	A	PO1	Improvement of the selectivity of Photodynamic Therapy (PDT): the « Photodynamic Molecular Beacons »
CHONGSHENG Qian	A	PO3	Adoptive Immunotherapy of refractory systemic adenovirus infections after allogeneic umbilical cord blood (UCB) or Peripheral Blood stem cell
PECHERY Adeline	A	PO4	Modulation of N-cadherin post-translational cleavage in bladder cancer cells
SCHMITT Antonin	A	PO5	Optimizing G-CSF Dosing Schedule to Prevent Eribulin-induced Neutropenia: Can Modelling & Simulation Help?
SABY Charles	A	PO6	Changes in extracellular matrix involving aging facilitate tumor cell proliferation
LAURENS Véronique	A	PO7	Glyceryl trinitrate inhibits secretion of clusterin and enhances cytotoxicity on docetaxel resistant prostate cancer cells
MIGNARD Caroline	A	PO8	In vivo TK-NOG liver-humanized model to predict patient pharmacological profile of anti-cancer agents
COLIAT Pierre	A	PO9	EGFR inhibition and radiotherapy induce an HIF-2 addiction in Head and Neck Cancer
MOREL Adrien	A	PO10	Epigenetic regulation of HPV16 E6
PLENCHETTE Stéphanie	A	PO11	New insights into glyceryl trinitrate (GTN) antitumor effect: GTN synergizes with TNFalpha to promote tumor cell death
DERANGERE Valentin	A	PO12	Modélisation in vitro de chimiothérapie intrapéritonéale hypotonique
MERLIN Jean-Louis	B	PO13	Detection of rare somatic mutational profiles in metastatic colorectal cancer (mCRC) during routine RAS next generation sequencing (NGS)
AMIOT Clotilde	B	PO15	Detection of leukemic cells in cryopreserved ovarian cortex using multicolor flow cytometry

DUBREZ Laurence	B	PO16	Analysis of the oncogenic properties of cellular Inhibitors of apoptosis (cIAPs)
BATTINI Stéphanie	B	PO18	Metabolome profiling by HRMAS NMR spectroscopy in parathyroid disorders
IMPERIALE Alessio	B	PO19	HRMAS NMR Metabolomics in pheochromocytomas and paragangliomas
CHAMARD-JOVENIN Clémence	B	PO20	Long chain alkylphenol mixture promotes breast cancer initiation and progression through an ERα36-mediated mechanism
BATTINI Stéphanie	B	PO22	Metabolome profiling by HRMAS NMR spectroscopy in pancreatic adenocarcinoma
BLANDIN Anne Florence	B	PO24	Implication of alpha5 beta1 integrin in resistance to anti-EGFR therapies in glioblastoma
TOURNIER Benjamin	B	PO25	Identification of an indirect prognostic DNA methylation marker highlights the importance of lncRNAs in stage II colon cancer
GARBAR Christian	B	PO26	MUC1- EGFR-NEU1 signalling pathway could be altered in triple-negative breast carcinoma and likely associated with resistance of EGFR-therapy.
PAUL Catherine	IT	PO27	A synthetic lipid A triggers colorectal tumor regression via a Neutrophil/G-MDSC-expressing granzyme B cell subset
PAUL Catherine	IT	PO28	Prognostic value of soluble FasL and TRAIL in patients with bladder cancer
PAUL Catherine	IT	PO29	Anticancer agents: does a phosphonium behave like a phosphine gold(I) complex? Let a "smart" probe answer!
DOSSET Magalie	IT	PO31	Tumor PD-L1 expression acts as an adaptative immune resistance mechanism to immunogenic cell death
MAUBANT Sylvie	IT	PO32	Evaluation of immuno-oncology related treatment in syngenic mouse models
GIUSTINIANI Jérôme	IT	PO33	Identification d'un nouvel anticorps monoclonal dirigé contre la mélanotransferrine exprimée par les cellules de mélanome
BENSUSSAN Armand	IT	PO34	Expression et fonctions des différentes isoformes de CD160 à la surface des lymphomes NK/T
HUDRY Delphine	IT	PO51	Follicular helper T cells in ovarian cancer
BURGY Olivier	IT	P052	Deglycosylated bleomycin, while keeping bleomycin's antitumor activity, lacks its pulmonary toxicity

BURCKEL Hélène	R	PO35	Comparison of ablative irradiation effects relative to those obtained with normofractionated irradiation
MIRJOLET Céline	R	PO36	Pre-clinical development of a Docetaxel nanocarrier to enhance prostate cancer radiosensitivity
LAURENT Carine	R	PO37	Toxicité des ions carbone en comparaison des rayons X dans des fibroblastes de peau
MASKALI Fatiha	I	PO40	Development of new [18F]-fluoro carbohydrate-based prosthetic groups and their conjugation to peptides via click chemistry
EL-BAKRI Adrian	I	P042	Nouvelles technologies de biophotonique cellulaire appliquées à l'oncologie
GAYDOU Vincent	I	P043	Infrared spectral diagnosis for predictive cancer medicine: application to the early diagnosis and prognosis of pre-invasive bronchial intraepithelial lesions
OJADA URIBE Mario	I	P046	Assessment of extramedullary hematopoiesis (emh) by hybrid imaging in patients with primary myelofibrosis (pmf)
ISAMBERT Nicolas	I	P047	Phase 0 clinical trials: A French patient's point of view
CHIEZE Lionel	I	P048	Investigating the influence of LRP-1 silencing by Atomic Force Microscopy reveals a promising non-invasive tool to establish a quantitative link between mechanical,
COLLIN Bertrand	I	P050	Nuclear imaging study of the effects of Debio 1143, a new oral SMAC mimetic inducing apoptosis in a triple-negative breast cancer model

PO1

IMPROVEMENT OF THE SELECTIVITY OF PHOTODYNAMIC THERAPY (PDT): THE « PHOTODYNAMIC MOLECULAR BEACONS ».

Auteurs et adresses : Aurélie Stallivieri1,2, Jérôme Devy3, Nicolas Etique3, Mathilde Achard2, Régis Vanderesse2 and Céline Frochot1
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Auteur présentant le résumé : Aurélie Stallivieri

Abstract :
One limitation of photodynamic therapy (PDT) is the low selectivity of photosensitizers to tumour tissue or neovascularization. Different strategies can be followed to improve the selectivity of the treatment such as bi-photon excitation or designing photosensitizers linked to specific vectors targeting membrane receptors overexpressed by cancer cells which becomes more and more a center of interest. Another interesting strategy is to produce reactive oxygen species specifically at the tumour site. It is this approach that fits our research. A promising approach is to use the activity of enzymatic cleavage of biomarkers overexpressed in tumour areas. Different enzymes such as matrix metalloproteinases (MMPs) are overexpressed in tumour development zones. Among these MMPs, gelatinases (MMP-2 and MMP-9) and membrane type 1 MMP (MT1-MMP) are known to play a key role in tumour angiogenesis [1, 2, 3] and the growth of many cancers such as glioblastoma multiform (GBM), an aggressive malignant tumor of the brain [4, 5]. We have the aim to synthesize and study photophysical properties and enzymatic action of photodynamic molecular beacons (PMBs), composed of a photosensitizer and a quencher linked together by a peptide substrate of gelatinases and MT1-MMP [6]. Our work consists in the synthesis and the photophysical study of different photosensitizer-peptide conjugates to understand the influence of 1) the nature and the position of the photosensitizer in the PMB, 2) the nature of the quencher on the enzymatic action. We will expose the synthesis strategy, the photophysical properties and the results of the enzymatic cleavage for the different derivatives and PMBs.
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PO3

ADOPTIVE IMMUNOTHERAPY OF REFRACTORY SYSTEMIC ADENOVIRUS INFECTIONS AFTER ALLOGENEIC UMBILICAL CORD BLOOD (UCB) OR PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

Auteurs et adresses : Chonseng Qian* 1, Véronique DECOT2, Yinying WANG1, Hui Li CAI3, Véronique VENARD4, Hélène JEULIN4, Marcelo DE CARVALHO BITTENCOURT3, Jean Hugues DALLE5, Cécile POCHON6, Bénédicte BRUNO7, Catherine PAILLARD8, Stéphane VIGOUROUX9, Charlotte JUBERT10, Philippe CEBALLOS11, Aude MARIE CARDINE12, Claire GALAMBRUN13, Laurence CLEMENT6, Danièle BENSOUSSAN1
1UTCT, Cell Therapy Unit , 2UTCT Cell Therapy Unit , 3Plateforme Nancytomique, 4Laboratoire de Virologie , CHU de Nancy, VANDOEUVRE LES NANCY, 5Service d'hémato-immunologie , Hôpital Robert Debré, Paris, 6UTMA , CHU de Nancy, VANDOEUVRE LES NANCY, 7Service d'Hématologie pédiatrique CHU de LILLE, CHU de LILLE, Lille, 8Service d'Hématologie Pédiatrique, CHU de Strasbourg, STRASBOURG, 9Service de transplantation médullaire, 10Servide d'Hématologie Pédiatrique, CHU de Bordeaux, BORDEAUX, 11Service de transplantation médullaire, CHU de Montpellier, MONTPELLIER, 12Service d'Hématologie Pédiatrique, CHU de Rouen, ROUEN, 13Service d'Hématologie Pédiatrique, CHU La Timone, MARSEILLE, France

Auteur présentant le résumé : Chongsheng QIAN

Abstract :
Introduction:Adenovirus (ADV) systemic infection refractory to antiviral treatment after allogeneic stem cell transplantation is associated with a high mortality rate up to 50%. Adoptive transfer of ADV-specific T cells (ADV-STs) is becoming an alternative treatment that has already proved feasible, safe and helpful in viral clearance and immune reconstitution related to an in vivo expansion of ADV-STs leading to clinical improvement.
Materials (or patients) and methods: We previously demonstrated the feasibility of isolating ADV-STs from healthy donors using the IFNg-capture system from Miltenyi followed by immunomagnetic selection on the CliniMACS device (Aissi-Rothé et al, 2010). We have now included 13 patients among 14 in a multicentric phase I/II clinical trial consisting in infusion of polyclonal ADV-STs generated by a 6-hour ex vivo stimulation with Pept-ADV5 hexon (peptide pool from the immunodominant Hexon protein, Miltenyi Biotec) of leukapheresis collected from their original stem cell donor or from third party haploidentical donors, followed by isolation of IFNg producing cells.
Results: We report on the generation of 12 ADV-ST preparations and the infusion of 10 pediatric and adult patients (8/2) with ADV infection (n=5; blood and/or stool viral load) or ADV disease (n=5). Five patients had experienced grade II GVHD controlled by immunosuppressive treatments before infusion. They received a mean of 5.84 103 CD3-IFNg+ cells/kg (range: 0,25 to 31.78 103) 1 to 9 months after HSCT. In vivo expansion of transferred ADV-STs was observed in 9 of 10 patients from day 14 to 60 following adoptive transfer infusion associated with ADV load decrease or clearance for 9 of them (90%) but one patient experienced a secondary increase. Neither de novo GVHD, nor side effects were observed. Reactivation of GVHD occurred in 3 patients and could be controlled by immunosuppressive treatments in 2 patients. In one patient, with stabilized GVHD grade II before infusion who experienced a modulation of immunosuppression before ADV-VSTs infusion, GVHD worsened in grade III with cutaneous, hepatic and intestinal manifestations responsible for the patient death. Two other patients died, one related to ADV disease (ADV and EBV meningitis), but none of the responders had ADV-associated mortality.
Conclusion: Adoptive transfer of ADV-STs is a feasible and well-tolerated therapeutic option, representing a fast and efficient procedure to achieve reconstitution of antiviral T-cell and decrease or clearance of ADV viral load. We expect that complete analysis of all the clinical data from the trial will allow attributing GVHD reactivations either to VSTs infusion or modulation of immunosuppressive drugs.

PO4

MODULATION OF N-CADHERIN POST-TRANSLATIONAL CLEAVAGE IN BLADDER CANCER CELLS

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Auteur présentant le résumé : Adeline Pechery

Abstract :
Despite the optimal treatments, including immunotherapy, chemotherapy and surgery, high grade non-muscle-invasive bladder cancer invading the mucosa has a high rate of recurrence and progression. Thus, the identification of reliable prognostic factors to predict aggressive potential of the disease is crucial for better treatment outcomes and patient quality of life.
In a previous study, the cell adhesion molecule N-cadherin has been identified as a prognostic progression marker. As it promotes tumor cell survival, migration, metastasis and contributes to angiogenesis, it has emerged as a potential therapeutic target for several cancers. Pharmacological inhibitors of N-cadherin could stop tumor development and prevent metastasis formation. This transmembrane glycoprotein is regulated at the post-translational level through proteases-mediated cleavage. ADAM10 is responsible for the initial proteolytic processing of N-cadherin leading to the generation of an extracellular fragment NTF and a C-terminal fragment CTF1. CTF1 is further cleaved by the presenilin/ɣ-secretase complex producing the soluble fragment CTF2. NTF is reported to stimulate migration and to induce angiogenesis. CTF2 translocates into the nucleus and activates gene transcription.
In our study, the aim was to target the potential N-cadherin shedding in T24 cells, derived from a metastatic urothelial tumor expressing high N-cadherin level. Thus, the impact of GW501516, a selective agonist of the ligand-inducible transcription factor PPARβ, was investigated on N-cadherin cleavage. This molecule decreases cell proliferation in breast and skin cancers and inhibits cell migration in vascular smooth muscle cells.
In T24 proliferating cells, a time course of 25 µM GW501516 from 30 min to 24 h revealed a decrease of NTF level in conditioned media from 6 h compared to control cells. These results suggested that GW501516 could decrease ADAM10 activity and consequently led to the inhibition of the ɣ-secretase activity which was validated by the accumulation of CTF1 during the time. Indeed, ADAM10-mediated N-cadherin ectodomain shedding is a prerequisite for the subsequent ɣ-secretase-induced cleavage. At 24 h-treatment of GW501516, a decrease of N-cadherin full length level was detected and this was associated with T24 cell apoptosis. The underlying mechanisms involved a mitochondrial membrane depolarization, the activation of caspases 8, 9, 3, the DNA fragmentation and a chromatin condensation.
To conclude, GW501516 could be a novel antitumor therapeutic agent targeting ADAM10. The regulation of ADAM10-mediated N-cadherin shedding in urothelial cancer could inhibit the invasive and migratory capacities of cells and thus the development of metastases. It is also important to clarify the biological role of both NTF and CTF N-cadherin fragments in urothelial malignant cells.

PO5

OPTIMIZING G-CSF DOSING SCHEDULE TO PREVENT ERIBULIN-INDUCED NEUTROPENIA: CAN MODELLING & SIMULATION HELP?

Auteurs et adresses : Pauline Macaire(1), Maria Inês Pereira(1), Isabelle Desmoulins(2), Nicolas Isambert(2), Bruno Coudert(2), Pierre Fumoleau(2), Antonin Schmitt(1, 2)
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Auteur présentant le résumé : Antonin Schmitt

Abstract :
Purpose:
Eribulin is a microtubule inhibitor indicated for the treatment of patients with metastatic breast cancer and should be administered on day 1 and 8 of each 21-day cycle. Neutropenia is one of its major side effects and can lead to delay or interruption of chemotherapy. Non-pegylated granulocyte colony stimulating factor (G-CSF) can be administered in order to prevent or treat chemotherapy-induced neutropenia. The aim of this study is, based on simulation, to describe the efficacy of G-CSF treatment on chemotherapy-induced neutropenia and propose an optimal dosing regimen for G-CSF administration in weekly-treated patients.
Material and Methods:
One thousand simulations were run for several G-CSF dosing schedules based on previous published models for eribulin treated patients (2.45 mg). The number of patients who experienced grade 3 and grade 4 neutropenia was calculated for each dosing schedule and also the duration of neutropenia.
Results:
G-CSF administration had significant beneficial effects for most dosing schedules. However, some dosing schedules revealed no overall benefits in preventing or treating chemotherapy-induced neutropenia by comparison with absence of G-CSF. Indeed, 43% and 22% of patients with G-CSF administered from day 3 to day 7 (1) experienced a grade 3 and 4 neutropenia, respectively. On the other side, a plan including administration of G-CSF from day 3 to 5 and then day 10 to 12 (2) turns out to be the best dosing schedule with respectively 31% and 10% of grade 3 and 4 neutropenia. Those results should be compared to respectively 50% and 18% of grade 3 and 4 neutropenia when no G-CSF was given (and 40% and 12% in the same situation, but with a 25% decreased eribulin dose).
With regards to duration, focusing on grade 3 neutropenia, the mean duration were respectively 8 and 3 days for G-CSF dosing schedule (1) and (2) (to be compared to 6 days without G-CSF). Moreover, when eribulin dose was reduced by 25 or 50 % in order to avoid G-CSF administration, there was no impact on neutropenia duration as compared to full dose.
Conclusion:
Our study demonstrates that, in weekly chemotherapy such as eribulin, the optimal G-CSF dosing schedule is during 3 days after each administration. We also underlined for the 1st time the potential negative effect of misused G-CSF. Those results need to be confirmed by a clinical trial, specifically in order to understand the high interindividual variability.

PO6

CHANGES IN EXTRACELLULAR MATRIX INVOLVING AGING FACILITATE TUMOR CELL PROLIFERATION

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Auteur présentant le résumé : Charles SABY

Abstract :
During cancer progression, the complex crosstalk between tumor cells and extracellular matrix (ECM) proteins is known to drive several main cellular functions such as proliferation. One important component of ECM proteins is fibrillar type I collagen that constitutes up to 90% of protein content in interstitial tissues. Interestingly, due to its particular longevity, with an estimated half-life of 15 years in humans, this key matrix protein happens to be a preferential target for non-enzymatic post-translational modifications during aging, such as glycation, that leads to Advanced Glycation End products formation (AGEs) and cross-linking. These modifications are cumulative and irreversible and lead to a loss in collagen fibrillar organization.
In this context, our study focused on the effect of aging on the interaction between tumor cells and collagen and its impact on human HT-1080 fibrosarcoma cell proliferation in a 3D matrix model. To this end, type I collagen was extracted from tail tendons of young-adult (2-month-old) and old (2-year-old) rats. In 3D matrices, the rate of HT-1080 cell growth was significantly lower in young-adult collagen when compared to the old one. This effect was accompanied by a down-regulation of the kinases ERK1/2 and JAK2 phosphorylation and an increase in the cyclin-dependent kinase inhibitor p21CIP1 expression. Experimental data permitted to exclude the involvement of β1 integrin and AGEs receptor (RAGE) in this process.
Accumulating evidence suggests that Discoidin Domain Receptor 2 (DDR2) is a receptor tyrosine kinase with the unique ability to be activated only by fibrillar collagen. Using siRNA strategy against DDR2, we have demonstrated that DDR2 depletion increases cell proliferation in young-adult collagen. To determine whether differential DDR2 responses are elicited by the two collagens, DDR2 was immunoprecipitated from cell lysates and its phosphorylated form was detected by immunoblotting. As expected, DDR2 phosphorylation level was increased in young-adult collagen matrices. This was accompanied by an increase in the tyrosine phosphatase SHP2 phosphorylation, the primary target of DDR2. In addition, DDR2 inhibition was also able to increase ERK1/2 / JAK2 phosphorylation and to decrease p21CIP1 expression.
To summarize, 3D matrix of old type I collagen significantly facilitates tumor cell proliferation compared to the young-adult one. Since DDR2 is well-known to be activated by fibrillar collagen, our data suggest that the increase in tumor cell growth is due to a lack of old type I collagen/DDR2 interaction. Finally, it also underlines the importance of age-mediated ECM structural changes that may influence tumor cell behavior.

PO7

GLYCERYL TRINITRATE INHIBITS SECRETION OF CLUSTERIN AND ENHANCES CYTOTOXICITY ON DOCETAXEL RESISTANT PROSTATE CANCER CELLS

Auteurs et adresses : Sarra Bouaouiche (1), Lucile Dondaine (1), Léa Magadoux (1), Anaïs Adolle (1), Stéphanie Plenchette-Colas (1), Nicolas Isambert (2), Jean-François Jeannin (1), Ali Bettaieb (1) et Véronique Laurens (1)
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Auteur présentant le résumé : Sarra Bouaouiche

Abstract :
Standard treatment of metastatic hormone-resistant prostate cancer (CRPC) is docetaxel chemotherapy. Nevertheless an escape is frequently observed to this chemotherapy following the acquisition of resistance by tumor cells. To overcome the emergence of chemotherapy resistance docetaxel combination therapies are evaluated. Since nitric oxide (NO) can modify the death sensibility statue of cancer cells, we study the cytotoxic efficacy of glyceryl trinitrate (GTN), a nitric oxide donor on CRCP cells sensitive or resistant to docetaxel. Moreover, the effect of GTN on regulation of clusterin, a chaperone molecule implied on docetaxel resistance is precise.
Two models of CRCP human cancer cells sensitive or resistant to docetaxel are used, DU-145 and PC3 parental cells and docetaxel resistant derived cells, DU-145 DR and PC3-D12 cells, respectively. The survival of these cells after treatment with GTN is evaluated in vitro by different tests (adhesion test, semi-solid colony culture, cell proliferation assay, flow cytometry). The expression of secreted and nuclear forms of clusterin are followed by Western blotting and ELISA, and the regulation of clusterin expression by q-RT-PCR. The action of GTN on these cell lines is also evaluated after transfection with siRNA clusterin. In addition, in vivo regulation of clusterin by GTN in DU-145 DR cells was assessed by q-RT-PCR using a xenograft approach in zebrafish larvae.
We demonstrate that docetaxel resistant cells are more sensitive to the cytotoxic effect of GTN than the parental cancer cell lines. Co-treatment of these human prostate cancer cells with docetaxel and GTN increases cell death. We observe that GTN decreases the expression of the secreted form of clusterin, which is the prosurvival isoform of clusterin, and that this regulation is implied in the cytotoxic effect of GTN. In addition, we check in vivo in a model of DU-145 DR cells xenografts in zebrafish larvae that GTN inhibits the synthesis of clusterin and reduces the growth of tumor cells. Our results show the benefits of a treatment with GTN capable of reducing the expression and secretion of clusterin and sensibilizing docetaxel resistant cells to cell death. In the future, combination treatment of docetaxel with GTN could be evaluated on CRCP patients in order to avoid emergence of docetaxel resistance cancer cells.

PO8

IN VIVO TK-NOG LIVER-HUMANIZED MODEL TO PREDICT PATIENT PHARMACOLOGICAL PROFILE OF ANTI-CANCER AGENTS

Auteurs et adresses : C. MIGNARD(1), O.DUCHAMP(1), F. NEMATI(2), N. CASSOUX (2), S. ROMAN-ROMAN(2), Y. OHNISHI (3), H. SUEMIZU(4)
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Auteur présentant le résumé : Caroline Mignard

Abstract :
To overcome some limitations of existing models, CIEA developed a novel experimental in vivo liver-humanized model. To do this, a herpes simplex virus type 1 thymidine kinase (HSVtk) transgene was expressed within the liver of highly immunodeficient NOG mice (TK-NOG). Mouse liver cells expressing this transgene were ablated after a brief exposure to a non-toxic dose of ganciclovir (GCV), and transplanted human liver cells are stably maintained within the liver (humanized TK-NOG) without exogenous drug. We have shown that the reconstituted liver is mature and functional and could generate:
A human-specific profile of anti-cancer drug metabolism. The humanization of the liver of TK-NOG mice modified the pharmacokinetic profile of the sorafenib anti-cancer agent. We were also able to detect the N-oxide metabolite of the sorafenib in humanized mice with a ratio of 8% of the non-metabolized sorafenib, in comparison to a 10% ratio in patients and 0% (not detectable) in non-humanized mice.
An efficient environment for metastatic cell homing in patient-derived-xenograft (PDX) model of UVeal melanoma. In two PDX UVeal melanoma models orthotopically xenografted in liver-humanized TK-NOG mice, we were able to detect liver metastasis, ranging from 10 to 50% of animals, whereas metastases have never been detected in non-humanized mice.
This novel in vivo system provides an optimized platform for increasing our predictivity of patient anti-cancer drug metabolism, potential toxicology, and efficacy.

PO9

EGFR INHIBITION AND RADIOTHERAPY INDUCE AN HIF-2 ADDICTION IN HEAD AND NECK CANCER

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Auteur présentant le résumé : Pierre COLIAT

Abstract :
Backround: Therapeutic management of Head and Neck Squamous Cell Carcinoma (HNSCC) are mainly based on surgery and chemo/radiotherapy. Treatments that target the Epidermal Growth Factor Receptor (EGFR) with the Cetuximab monoclonal antibody are the only targeted therapies approved for management of locally advanced HNSCC. However, the prognosis has not improved in the last decades, with less than 50% of the patients being alive 5 years after treatment. The resistance of these tumors to treatment might involve tumor hypoxia. Indeed, it has been shown that the stabilization of Hypoxia Inducible Factors (HIF), that are key regulators of the cell adaptation to hypoxia, correlates with tumor resistance to ionizing radiations and adverse prognosis. HIF-1α expression can be induced independently of oxygen concentration by oncogenic signaling pathways, like the EGFR/mTOR pathway.
Pharmacological approaches using targeted agents that inhibit the EGFR/mTOR pathway with specific inhibitors have shown efficacy in some solid tumors such as Non Small Lung Cells Cancer. Moreover, some studies suggest that the use of mTOR inhibitors sensitizes radioresistant cells and anti-EGFR resistant cells.
Methods: We xenografted the cetuximab-sensitive (CAL27) and cetuximab-resistant (SQ20B) cell lines to nude mice, and evaluated the impact of cetuximab and rapamycin, alone or in combination with radiation therapy, on HIF-1 expression, as well as on tumor growth and time to relapse. DNA double-strand breaks generation, clonogenic survival, and EGFR/mTOR axis signaling activation were evaluated in vitro.
Results:
The Rapamycine/Cetuximab combination showed a remarkable efficacy on tumour growth control in vivo. Rapamycin combined to EGFR-targeted treatment improves sensitivity of the resistant SQ20B cells lines to radiation therapy in vitro
However, radiotherapy combined to either cetuximab, or cetuximab + rapamycin resulted in shorter time to regrowth and increased relapse frequency, despite the fact that the drug combination and irradiation increased DNA double strand breaks. However, despite efficient HIF-1 inhibition, SQ20B clonogenic survival after treatment was only reduced to about, 50% suggesting an induced mechanism of adaptation. In consequence, we investigated if HIF-2 takes a part in this adapation. We observed HIF2 expression was induced by EGFR treatment and/or Irradiation.
HIF-2 silencing using specific SiRNA alone had no impact on clonogenic survival. However HIF-2 silencing combined with treatment induced a dramatic drop of clonogenic survival (<1% clonogenic survival).
Conclusion: HIF-2 oncogenic addiction is induced by radiotherapy and EGFR treatments and could be implicated in resistance and recurrence phenomenon in Head and Neck cancer patient.

PO10

EPIGENETIC REGULATION OF HPV16 E6

Auteurs et adresses : Morel Adrien 1, Jacquin Elise 2, Baguet Aurélie 1, Demeret Caroline 3, Hervouet Eric 4, Mougin Christiane 1, Prétet Jean-Luc 1.
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Auteur présentant le résumé : Adrien Morel

Abstract :
Background: High risks Human Papillomaviruses (HPV) are causative agent of cervical cancer and HPV16 is the most prevalent type. HPV genome consists in a ds circular DNA with two regions encoding: i) early "E" and late "L" proteins and ii) a Long Control Region (LCR) involved in viral cycle and early gene regulation. HPV DNA integration in the host cell genome, a frequent event in cervical carcinogenesis, leads to the loss of the E2 gene and consequently to E2 protein. E2 normally binds to E2 Binding Sites (E2BS) present on the LCR and represses transcription of E6 and E7 oncoproteins. Therefore, a loss of E2 induces an overexpression of E6 and E7 proteins that induce p53 and pRb degradation, and hTERT overexpression. Since CpG dinucleotides are present in HPV16 E2BS, we investigated whether E6 HPV16 expression is also submitted to epigenetic regulation.
Results: CaSki cells (integrated HPV16 DNA, methylated E2BS) were treated by 2.5 µM of 5-aza-2'-deoxycytidin (5azadC) for 48h and a decrease of E2BS methylation was observed. This was accompanied by a decrease of E6 expression both at the mRNA and protein levels. Furthermore, CaSki cells were transfected with pciNeoE2 to restore E2 expression. The decrease of E6 expression was even more significant when E2 was transfected in CaSki cells treated by 5azadC.
A chromatin immunoprecipitation (ChIP) assay with anti-E2 and control antibodies was then performed in CaSki cells transfected or not with peGFPE2 to determine whether E2BS demethylation promotes the binding of E2 on E2BS. We found that a 5azadC treatment of E2 expressing CaSki cells induced a 2-fold enrichment of E2BS containing fragments. This clearly indicates that viral DNA demethylation promoted the binding of E2 on the viral promoter.
Unexpectedly, a decrease expression of E6 was also observed in SiHa cells (integrated HPV16 DNA, unmethylated E2BS) treated by 5azadC. Thus, we hypothesized that cellular factors, themselves submitted to epigenetic regulation, could modulate viral oncoprotein expression. Among candidates, Sp1 able to bind Sp1BS also present on HPV16 promoter is unlikely to be involved as its expression is increased in both CaSki and SiHa cells treated by 5azadC. In contrast, TBX20, a transcriptional factor related to TBX2 himself known to repress HPV16 promoter, is a promising candidate as its expression is increased by 5azadC in both cell lines.
Conclusion: HPV16 E6 oncoprotein expression is regulated by E2 and by a cellular factor itself regulated in epigenetic manner. HPV-associated carcinogenesis is not yet fully understood and deciphering new molecular mechanisms involved in tumor development gives opportunities to identify new biomarkers and therapeutic targets.

PO11

NEW INSIGHTS INTO GLYCERYL TRINITRATE (GTN) ANTITUMOR EFFECT: GTN SYNERGIZES WITH TNFALPHA TO PROMOTE TUMOR CELL DEATH

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Auteur présentant le résumé : Sabrina Romagny

Abstract :
One of the key features of tumor cells is the acquisition of resistance to apoptosis. Current chemotherapeutic strategies aim at restoring cancer cell death but their efficacy is largely limited by acquired chemoresistance. Determining new therapeutic strategies that circumvent chemoresistance and result in tumor regression is a challenge. We propose a Nitric Oxide (NO)-based therapeutic approach using the GTN (Glyceryl Trinitrate) as a NO donor. Yet, antitumor effects of GTN have been demonstrated in two phase II clinical trials in patient with non-small cell lung cancer and relapsing prostate cancer (Yasuda et al. 2006; Siemens et al. 2009). NO, a highly reactive free radical, is emerging as a potent cancer cell death sensor in a wide variety of tumor cells. Our objective is to better understand the role of NO as a sensitizing agent to increase the effectiveness of cancer therapy.
We and others, showed that NO sensitizes cancer cells to death mediated by members of the Tumor Necrosis Factor (TNF)-family ligands (Fas and TRAIL). To further delineate the role of NO in the TNF-family signaling pathways, we have deciphered regulatory mechanism of the TNFalpha/TNFR1 signaling cell death pathway following GTN treatment. TNFR1, engaged by TNFalpha, can activate both cell proliferation and cell death. In this context, cell fate decision is determined by the ubiquitylation function of specific IAP (Inhibitor of APoptosis) proteins, cIAP1 and cIAP2. To mimic the effect of TNFalpha encountered by tumor cells in the tumor microenvironment, we have used recombinant TNFalpha or TNFalpha secreted by activated macrophages. We show that GTN in presence of TNFalpha sensitizes human and murine colonic and mammary tumor cells to apoptosis. Importantly, we demonstrate that chemotherapies (such as irinotecan) induce TNFalpha production by cancer and immune cells.
Mechanistically, the classical NF-κB signaling pathway is impaired following GTN treatment in presence of TNFalpha. Moreover, we show that GTN promotes cIAP1 S-nitrosylation by the Biotin Switch Assay. Mass spectrometry analyses identified critical sites of S-nitrosylation, and more particularly two sites in its ubiquitin-E3 ligase domain. It appears that NO affects cIAP1 enzymatic activity that initiates the critical switch to cell death. Our results suggest that GTN may exert a potent antitumor effect in presence of endogenous TNFalpha (that could be induced by chemotherapies) particularly into the tumor microenvironment. Thus, the combination of GTN with an appropriate chemotherapy, such as irinotecan, may represent an interesting anticancer strategy.

PO12

MODELISATION IN VITRO DE CHIMIOTHERAPIE INTRAPERITONEALE HYPOTONIQUE

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Auteur présentant le résumé : Valentin Derangère

Abstract :
La chimiothérapie intrapéritonéale hyperthermique (CHIP) est utilisée pour traiter les métastases intra-cavitaires d'origine colique ou ovarienne. La CHIP permet d'augmenter l'espérance de vie et la survie des patients souffrant de carcinose péritonéale. Les procédures de CHIP étant peu standardisées, nous modélisons actuellement in vitro cette technique chirurgicale afin d'évaluer le rôle des différents paramètres possibles de cette procédure, comme l'osmolarité du soluté, la température, le temps d'exposition et la chimiothérapie sur la viabilité cellulaire.
Nous montrons que les dérivés du platine, comme le cis-platine ou l'oxaliplatine lorsqu'ils sont instillés en conditions hypoosmolaires in vitro possèdent une cytotoxicité plus importante par rapport aux conditions isoosmolaires sur les cellules cancéreuses coliques humaines HCT116. Nous avons exploré les mécanismes de cytotoxicité et les voies moléculaires impliquées dans notre modèle.
Ces expériences permettront d'envisager des essais thérapeutiques chez des animaux souffrant de carcinoses péritonéales Ces travaux auront des retombées importantes, notamment en chirurgie digestive, en offrant une base de travail majeure pour les chirurgiens réalisant des CHIP. Nos travaux pourraient ainsi améliorer la réponse au traitement et la survie des patients atteints de carcinose péritonéale.

PO13

DETECTION OF RARE SOMATIC MUTATIONAL PROFILES IN METASTATIC COLORECTAL CANCER (MCRC) DURING ROUTINE RAS NEXT GENERATION SEQUENCING (NGS)

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Auteur présentant le résumé : Alexandre Harlé

Abstract :
Background: In most patients with mCRC who are being considered for anti-EGFR antibody therapy, RAS mutation testing i.e. KRAS and NRAS exon 2, 3 and 4, is routinely assessed using PCR-based assays only detecting major hotspot mutations of exon (ex) 2 (codon 12 and 13), 3 (codon 59 and 61) and 4 (codon 117 and 146). We performed deep sequencing of the entire exons using NGS as an alternative to detect additional rare mutations profiles with significant frequency of mutated allele (FMA). Methods: 188 formalin-fixed paraffin-embedded tumor samples from primary or metastatic lesions of patients (M/F sex ratio 1.27, mean age 69 years, range 32-90) with mCRC (150 colon, 38 rectum) were analyzed. DNA was extracted from macrodissected slides (mean tumor cell content 43.3%, range 5-80). Results: RAS mutation testing was routinely assessed using NGS in 177 mCRC samples. NGS could not be performed in 11 cases (6.2%) due to the insufficient quantity or quality of DNA. NGS sensitivity was 1% at X1000 depth. RAS mutations were found in 103 samples (62%) and relatively distributed as 69.9% KRAS ex2, 3.9% KRAS ex3, 14.6% KRAS ex4, 4.8% NRAS ex2, 1.0% NRAS ex3, 1.0% NRAS ex4 and 4.8% multiple mutations. Uncommon mutational profiles were detected in 10 cases (9.7%): 2 KRAS ex2 c.37G > T p.G13C single mutation with FMA > 30%, 5 silent mutations (4 with FMA > 25%), alone (n = 2) or combined with other rare mutations (n = 3) with lower but significant FMA (> 1%), and 6 multiple mutation profiles among which 2 double hotspot mutation (KRAS ex2 c.34G > A p.G12S and NRAS ex3 c.181C > A p.Q61K, KRAS ex2 c.34G > A p.G12S and NRAS ex2 c.38G > T p.G13V), 1 secondary rare mutation associated with a KRAS ex2 c.35G > A p.G12D hotspot mutation, and 3 multiple mutations only with rare but potentially deleterious mutations located around the loops responsible for nucleotide (GTP) binding. In only 1 case, the FMA of the secondary mutations was < 1%. As a whole, 7 cases (6.8%) had RAS mutations out of hotspots. Conclusions: NGS proved accurate, sensitive and suitable for routine RAS genotyping in mCRC. It can detect uncommon RAS mutation profiles with significant FMA that can potentially impair the patient response to anti-EGFR antibody.

PO15

DETECTION OF LEUKEMIC CELLS IN CRYOPRESERVED OVARIAN CORTEX USING MULTICOLOR FLOW CYTOMETRY

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Auteur présentant le résumé : Christophe Roux

Abstract :
Introduction:
Ovarian cortex cryopreservation performed before potentially sterilizing treatment and its secondary autograft, is a real option to preserve and then restore fertility. However in leukemia patients, there is a real concern regarding the presence of cancer cells in the cryopreserved ovarian cortical strips, which could lead to the recurrence of the disease when grafting. The purpose of this work was to develop and validate an original technique of minimal residual disease (MRD) detection in ovarian cortex from acute leukemia patients, using multicolor flow cytometry (MFC).
Material and Methods:
We developed an automated dissociation protocol for ovarian cortex, combining mechanical and enzymatic effects and we designed an experimental model consisting in the addition of a series of leukemic cell dilutions into isolated cell suspensions obtained from non contaminated ovarian cortex. MFC allowed us to identify leukemic cells by their specific leukemia associated phenotype (LAP), among the viable single cell subpopulation. This modelization was validated for acute lymphoblastic leukemia (ALL) and also for acute myeloid leukemia (AML). Then the method was applied to MRD detection in cryopreserved ovarian cortex from 7 ALL and 4 AML patients.
Results:
In this experimental model, we are able to detect the presence of leukemic cells by MFC with a high specificity and a robust sensitivity of 10-4 (20 events with specific LAP among 200 000 viable events). Of the 11 leukemia patients tested, one T-ALL and 2 AML patients showed a positive ovarian MRD by MFC, while no molecular markers were available for these 3 patients.
Conclusion:
MFC is a useful method for MRD detection in ovarian cortex in leukemia patients. This approach can be applied to 100% of acute leukemia patients and is essential (used alone or associated with PCR and/or xenograft) to evaluate the risk of cancer reseeding before ovarian cortex autograft.

PO16

ANALYSIS OF THE ONCOGENIC PROPERTIES OF CELLULAR INHIBITORS OF APOPTOSIS (CIAPS)

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Auteur présentant le résumé : Jennifer Allègre et Laurence Dubrez

Abstract :
Cellular Inhibitors of Apoptosis (cIAP1 and cIAP2) belong to the Inhibitor of APoptosis (IAP) family which contains eight mammal members. Their expression has been shown to be altered in number of tumor samples and oncogenic properties have been demonstrated in mice. cIAP-encoding genes are located on a chromosome region which appeared amplified in several cancers (amplicon 11q22). cIAPs are cell signaling molecules regulating several cellular processes which can contribute to tumor development including inflammatory and immune response, cell death, cell proliferation and cell migration. Therefore, targeting IAPs is a great interest for cancer therapy and several IAP antagonists have been designed and are currently evaluating in clinical trials. The understanding of the molecular mechanisms responsible for the oncogenic activity could help to the design of function-specific inhibitors in order to minimize secondary effects. cIAPs are endowed with E3-ubiquitine ligase activity. They can interact with several protein partners thanks to the presence of 3 protein-protein interacting domains named BIRs (baculoviral IAP repeats) and they catalyze the conjugation of ubiquitin chains onto partners because of the presence of a C-terminal Ring domain.
In order to analysis oncogenic properties of cIAPs, cIAP1 or cIAP1 and cIAP2-deficient tumor cell lines have been established by expressing H-Ras-V12 oncogene into cIAP1 or cIAP1 and cIAP2-depleted fibroblasts. H-Ras transformed, cIAP1-deficient-cells display a decrease in clonogenic capacity, in adhesion properties and in the capacity to intercalate between endothelial cell monolayer compared to the control conterpart. In nude mice, deficiency of cIAP1 significantly inhibited the growth of tumour cells when subcutaneously injected and delayed the apparition of lung cancer foci after injection of cells into the tail vein. Deletion of both cIAP1 and cIAP2 emphasizes the effects. cIAP-deficient tumor cell lines were restored with cIAP1 or several cIAP1 mutants and analysed for their oncogenic properties. Expression of cIAP1 or cIAP1 mutants all restored the tumor growth in xenograft model. However, while expression of cIAP1 or a cIAP1 mutant lacking the second BIR domain able to interact with actin cytoskeleton regulators restored the capacity of cell to colonize lung, a H588A mutation which abolishes the E3-ubiquitine ligase activity of cIAP1 or the deletion of the first BIR domain which interact with the tumor necrosis factor-associated cell signalling molecules inhibited oncogenic properties of cIAP1. Overall, our results suggest that the E3-ubiquitine ligase activity of cIAP1 and its capacity to control TNF-signalling pathways are important for its oncogenic activity.

PO18

METABOLOME PROFILING BY HRMAS NMR SPECTROSCOPY IN PARATHYROID DISORDERS

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Auteur présentant le résumé : Stéphanie Battini

Abstract :
Abstract:
Introduction The aim of the present study was to compare the metabolomic profiles of parathyroid disorders of different origins (primary, secondary and tertiary hyperparathyroidism, "HPT").
Materials and methods We used frozen tissues for all sample preparations. All High Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HRMAS NMR) spectra were achieved on a Bruker Avance III 500 spectrometer which operated at a proton frequency of 500.13 MHz. A one-dimensional (1D) proton spectrum was acquired for each biopsy's insert. A combination of Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) was then adopted to evaluate the quality of our data, to identify possible outliers and to optimize the separation between tumor subgroups.
Results Significant differences in metabolomic profiles were assessed according to both pathologic diagnosis (adenoma, hyperplasia) and HPT type. Our preliminary results show that HRMAS NMR spectroscopy is a reliable method for classifying hyperfunctioning parathyroid lesions in patients with HPT. Moreover, we can notice that the metabolomic profile of parathyroid glands seems to be less influenced by histologic origin than by HPT type, allowing for the existence of complex physiopathological mechanisms.
Conclusion/discussion The present study shows that HRMAS NMR could provide some new information about the characterization of hyperfunctioning parathyroid glands according to both histologic origin and HPT type. These findings might have both clinical and biological implications. However, the real impact of these interesting results should be brought out in long-term prospective studies performed in a larger cohort of patients.

PO19

HRMAS NMR METABOLOMICS IN PHEOCHROMOCYTOMAS AND PARAGANGLIOMAS

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Auteur présentant le résumé : Stéphanie Battini

Abstract :
Introduction Pheochromocytomas/paragangliomas (PHEOs/PGLs) are characterized by high genetic heterogeneity. Mutations in succinate dehydrogenase (SDH) genes (SDHx) increase susceptibility to develop PHEOs/PGLs. The SDHx genes encode the SDH enzyme that catalyzes the oxidation of succinate to fumarate in the tricarboxylic acid cycle and the respiratory chain. The aim of the present study was to define the global metabolomic profile of the SDH-related PHEOs/PGLs in comparison to sporadic tumors and, identify metabolites that could be used as clinical

predictors of SDH deficiency using High Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HRMAS NMR) spectrosocpy.
Materials and methods We used frozen tissues for all sample preparations. All HRMAS NMR spectra were achieved on a Bruker Avance III 500 spectrometer which operated at a proton frequency of 500.13 MHz. A one-dimensional proton spectrum was acquired for each biopsy's insert. A combination of Principal Component Analysis and Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) was then adopted to evaluate the quality of our data, to identify possible outliers and to optimize the separation between tumor subgroups.
Results A two-component OPLS-DA showed a very clear separation between sporadic and SDHx-related tumors. Compared to sporadic, SDHx-related PHEOs/PGLs exhibit a specific metabolic signature characterized by increased levels of succinate ($p<0.0001$), methionine ($p=0.002$), glutamine ($p=0.002$) and myo-inositol ($p<0.0007$) and decreased levels of glutamate ($p<0.0007$), regardless of their location and catecholamine levels. Uniquely, ATP/ascorbate/GSH were found to be associated with the secretory phenotype of PHEOs/PGLs, regardless of their genotype ($p<0.0007$). The use of succinate as single screening test retained excellent accuracy in distinguishing SDHx vs. non-SDHx-related tumors (Se/Sp: 100/100%, cut-off value: 0.096 nmol/mg). ROC curves were also built using the quantitative values of glutamate, methionine, myo-inositol and methionine/glutamate ratio. However, the diagnostic accuracy obtained for each single metabolite was lower than the accuracy achieved by succinate as a single tumor biomarker. When the data is analyzed using the ADEMA algorithm the results showed that network and mutual information based analysis is in accordance with the statistical significance of changes shown above, with the exception of aspartate.
Conclusion/discussion The present study shows that HRMAS NMR spectroscopy is a very reliable method for classifying various PHEOs/PGLs according to their genetic background. Our work well justifies that, in the near future, functional genomics will allow for and perfect the identification of tumor-specific metabolic biomarkers as well as their genetics. It is expected that cancer metabolome will be quickly implemented in new diagnostic and treatment options of various cancers as well as their prognosis.

PO20

LONG CHAIN ALKYLPHENOL MIXTURE PROMOTES BREAST CANCER INITIATION AND PROGRESSION THROUGH AN ERA36-MEDIATED MECHANISM

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* Both authors co-supervised the work.

Auteur présentant le résumé : Clémence Chamard-Jovenin

Abstract :
Background : Growing source of evidence suggests that exposure to estrogen mimicking agents is a risk factor for breast cancer onset and progression. Long chain alkylphenols are man-made compounds still present in industrial and agricultural processes. Their main use is domestic and they are widespread in household products, cleansers and cosmetics, leading to a global environmental and human contamination. These molecules are known to exert estrogen-like activities through binding to classical estrogen receptors. Recently, we have demonstrated that a realistic mixture of 4-tert-octylphenol and 4-nonylphenol can stimulate proliferation and modulate epigenetic status of testicular cancer germ cells through a rapid, Estrogen Receptor alpha 36 (ERα36) - phosphatidyl-inositol 3-kinase (PI3-kinase) non genomic pathway.
Since expression of ERα36, a variant of the canonical Estrogen Receptor alpha (ERα66) was shown to mediate mitogenic signal, stimulate migration and was described as a marker of poor prognosis in breast tumors, we addressed the question of its involvement in response to alkylphenol exposure in MCF10A mammary epithelial cell line and MCF7 estrogen-sensitive cancer cells.
Methods : ERα36 overexpression or gene-silencing strategies combined to microarray analyses of the mixture target genes were used in MCF10A and MCF7 cells in order to characterize the molecular phenotype of exposed cells. A customized database was designed to analyze comprehensive gene expression results, nonlinear correlation analyses, and mutual information computations helpful for the modeling of ERα36-dependent pathways.
Results: Our results highlight a key role for ERα36 in alkylphenol non genomic src protein kinase / PI3-kinase / serine-threonine kinase Akt / nuclear factor-kappa B signaling in non cancerous epithelial breast cells. Hence, alkylphenol and/or ERα36-dependent control of the proliferation, adhesion and survival pathway opens the way for understanding the link between endocrine disruptor exposure and the burden of hormone sensitive cancers.
Conclusion: This study indicates that ERα36 could represent a key node into a novel pathway involved in metaplastic transformation of breast epithelium during tumor initiation and cancer progression.

PO22

METABOLOME PROFILING BY HRMAS NMR SPECTROSCOPY IN PANCREATIC ADENOCARCINOMA

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Auteur présentant le résumé : Stéphanie Battini

Abstract :
Introduction The aim of the present study was to define the global metabolomic profile of the pancreatic adenocarcinoma in comparison with healthy pancreatic tissue. The main idea was to identify metabolites that could be used as biomarkers using High Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HRMAS NMR) spectroscopy.
Materials and methods
Sixty-five samples were included in this study (thirty-nine pancreatic adenocarcinoma and twenty-six healthy pancreatic tissue). We used frozen tissues for all sample preparations. All HRMAS NMR spectra were performed on a Bruker Avance III 500 spectrometer which operated at a proton frequency of 500.13 MHz. A one-dimensional (1D) proton spectrum was acquired for each biopsy's insert. A combination of Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) was then adopted to evaluate the quality of our data, to identify possible outliers and to optimize the separation between tumor subgroups.
Results PLS-DA distinguishes pancreatic adenocarcinoma from healthy pancreatic tissue (R2Y = 0.85, Q2= 0.66). Significant differences in metabolomic profiles were highlighted. Our preliminary results show that HRMAS NMR spectroscopy is a reliable method for classifying pancreatic lesions in patients suffering from pancreatic adenocarcinoma. Indeed, the metabolome profile in pancreatic adenocarcinoma differs from metabolome profile in healthy pancreatic tissue. Healthy pancreatic tissue exhibit a specific metabolic signature characterized by increased levels of glucose, glycine and, myo-inositol. Increased levels of ascorbate, glycerophosphocholine, phosphorylcholine, taurine and aspartate were highlighted in pancreatic adenocarcinoma.
Conclusion/discussion The present study demonstrates that HRMAS NMR spectroscopy provides important and solid information in the characterization of pancreatic adenocarcinoma. Metabolomic profiles inspection lead to potential biomarkers or oncometabolites identification involved in the pathogenesis of different entities. These findings might have clinical and biological implications.

PO24

IMPLICATION OF ALPHA5 BETA1 INTEGRIN IN RESISTANCE TO ANTI-EGFR THERAPIES IN GLIOBLASTOMA

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Auteur présentant le résumé : Anne-Florence Blandin

Abstract :
Glioblastoma multiforme (GBM) is the most common primary brain tumor. Amplification and mutation of the epidermal growth factor receptor (EGFR) is detected in about 50% of patients with GBM. Clinical trials using anti-EGFR therapies for the treatment of GBM reveal limited efficacy. Our team previously showed that overexpression of the fibronectin receptor, α5β1 integrin, is associated with a poor prognosis for patients and that the integrin triggers resistance to chemotherapy. Moreover, integrins can cross-talk with tyrosine kinase growth factor receptors to promote cell growth survival and migration. The aim of my thesis is to characterize the functional interaction between EGFR and α5β1 integrin in GBM cells and determine its potential involvement in resistance to anti-EGFR targeted therapy.
We genetically modified a GBM cell line, to overexpress (U87-α5+) or repress (U87-α5-) α5 integrin expression. We first examined the impact of EGFR/a5 crosstalk on cell migration with anti-EGFR drugs (Cetuximab®- CTX and Gefitinib®- GEF) used in clinical trials. Using Boyden chamber assay with a fibronectin coating, we showed that U87-α5+ cells are resistants to CTX and GEF activity. By contrast, the loss of α5 integrin sensitizes U87 MG cells towards anti-EGFR drugs. Inhibition of α5 integrin with specifics inhibitors (RGD mimetic antagonist and antibody) restores the sensitivity of U87-α5+ cells toward CTX demonstrating the importance of a5 integrin in resistance to anti-EGFR drugs in GBM. Next, we examined the impact of α5 integrin expression on collective cell migration from spheroids. We examined the propensity of cells to migrate out of spheroids onto fibronectin coating. The addition of GEF during cell migration significantly reduces U87-α5- cell migration (50% vs control). By contrast, U87-α5+ cells are totally resistant to anti-migratory activity of GEF. EGFR and α5 integrin trafficking are crucial events during cell migration and invasion. Using confocal microscopy, we showed that GEF treatment induces a profound translocation of α5β1 integrin from fibrillar adhesions to intracellular vesicles which are not identified for the moment. Moreover, GEF differently affected EGFR localization in U87-α5+ spheroids and in U87-α5- spheroids.
Using two models of migration, we showed that α5 integrin drives U87-MG cells resistance to anti-EGFR drugs. A drug association targeted EGFR and α5β1 might be a new therapeutic option to overcome resistance in brain tumors which overexpress α5β1 integrin. Moreover, GEF treatment altered α5β1 integrin localisation suggested that regulation of EGFR trafficking by the integrin may be implicated in resistance to anti-EGFR drugs in GBM cells.

PO25

IDENTIFICATION OF AN INDIRECT PROGNOSTIC DNA METHYLATION MARKER HIGHLIGHTS THE IMPORTANCE OF LNCRNAS IN STAGE II COLON CANCER

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Abstract :
Colorectal cancer is still a major public health problem as it represents the third leading cause of cancer death in the world. Currently, the most powerful tool to adapt the treatment of patients is the TNM grading system. This system is not optimal. For stage II colon cancers, which have no node involvement, the current recommendations are to perform surgery alone. However, around 25% of these patients relapse and die from their cancer. The TNM classification needs to be completed with bio-markers that could help to identify cases with a worse outcome, in order to adjust the treatment. Molecular alterations seem appropriate for such task.
As cancer cells have the potential to de-differentiate and then re-differentiate, it means that some capabilities are not lost but transiently extinct. Perturbations in mechanisms involved in gene expression are therefore of great interest for the study of cancer development. Gene expression is partly controlled by epigenetic mechanisms, including DNA methylation, which is the most referenced. In colon cancer, many perturbations in the DNA methylation profile have been reported: a decrease in the 5-methy-cytosine content, termed global DNA hypomethylation; and a specific acquisition of DNA methylation marks in the promoter regions of genes, called the CpG island methylator phenotype (CIMP). The purpose of this study was to identify prognostic markers deregulated by DNA methylation changes in the context of colon cancer.
From a cohort of 375 sporadic stage II colon cancer cases, for which we had both cryo-preserved tissues and clinical data from patients, we carried out a methylome analysis using DNA methylation array based on Illumina GoldenGate technology. Among the 1505 CpG sites analysed, we identified CpG sites demonstrating DNA methylation level variations that were associated with significantly worse overall survival. One of these was located in the promoter region of the CD81 gene. We found a correlation between the methylation profile of CD81 promoter and CD81 RNA expression in colon cancer cell lines, but the protein was not detectable by immunohistochemistry in the tumour tissues. Further investigations showed that long intergenic non-coding RNAs (lincRNA) were also possibly transcribed from these regions and could be affected by the methylation status of this CpG site.
CD81 methylation seems to be an indirect prognostic marker and the mechanism involved could be the repression of lincRNA at the vicinity of CD81. Our objective is now to investigate the link between methylation of this site and expression of the corresponding lincRNAs, and also to understand the role of these lincRNAs. The detection of these types of non-coding RNAs from tumour biopsies or blood of patients could open the way to a new type of biomarkers.

PO26

MUC1- EGFR-NEU1 SIGNALLING PATHWAY COULD BE ALTERED IN TRIPLE-NEGATIVE BREAST CARCINOMA AND LIKELY ASSOCIATED WITH RESISTANCE OF EGFR-THERAPY.

Auteurs et adresses : Christian Garbar (1,2), Corinne Mascaux (1,2), Jérôme Giustiniani (1,2), Stéphanie Salesse (3),Laurent Debelle (3), Frank Antonicelli (2), Yacine Merrouche (1,2), Armand Bensussan (4)
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Auteur présentant le résumé : Christian Garbar

Abstract :
Triple-negative breast carcinoma (TN) is a heterogeneous cancer type expressing EGFR in 75% of cases. Unfortunately, the treatments by a monoclonal anti-EGFR alone (Cetuximab) or in combination with carboplatin, were associated with a low rate of clinical response suggesting a complex signalling pathway.
MUC1 is a large type I sialylated glycoprotein comprising two subunits (α and β chains, also called respectively MUC1-VNTR and MUC1-CT), which was found to regulate EGFR activity through endocytic internalisation. Endocytosis and autophagy use the lysosome pathway involving NEU1. Recently, a molecular EGFR-MUC1-NEU1 complex was suggested to play a role in EGFR pathway. In the aim to understand EGFR-MUC1-NEU1 molecular complex in breast carcinoma, we compared triple negative (TN) showing a high-EGFR expression with luminal (LUM) presenting low-EGFR level. We studied the expression of MUC1-VNTR, MUC1-CT and NEU1 in comparison with those of two molecular actors of autophagy involved in endocytosis, PI3K (p110β) and Beclin1. A total of 87 breast cancers were split in two groups following the immunohistochemical classification of breast carcinoma: 48 TN and 39 LUM. Our results showed that TN presented a high expression of EGFR and a low expression of MUC1-VNTR, MUC1-CT, NEU1, Beclin-1 and PI3Kp110β. Moreover, in TN, a positive statistical correlation was observed between Beclin-1 or PI3Kp110β and MUC1-VNTR or NEU1, but not with EGFR. In conclusion, our data suggest that autophagy could be reduced in TN leading likely to the deregulation of EGFR-MUC1-NEU1 complex.

PO27

A SYNTHETIC LIPID A TRIGGERS COLORECTAL TUMOR REGRESSION VIA A NEUTROPHIL/G-MDSC-EXPRESSING GRANZYME B CELL SUBSET

Auteurs et adresses : Amandine Martin*1, Cédric Seignez*1, Nadhir Yousfi, Cindy Godard1, Lucile Dondaine1, Alessandra Scagliarini1, Nicolas Isambert1,2, Arlette Hammann3, Amandine Bataille4, Laurent Arnould2, Ali Bettaieb1, Jean-François Jeannin1, Catherine Paul1, 5

Auteur présentant le résumé : Nadhir Yousfi

Abstract :
We previously reported that a synthetic lipid A induced tumor regression in rat model of colon carcinoma and was safe in human. Here, we confirm this effect in a mouse model of colon carcinoma and demonstrate that the mechanisms of tumor regression by this lipid A require innate immune response. Initiated by lipid A, tumor infiltration by Neutrophil/G-MDSC cells, which was correlated with intratumoral increase of specific chemokines, was necessary for tumor regression. Phenotypic and functional analyses demonstrated that (a) unexpectedly, Neutrophil/G-MDSC cells, from tumors but not from spleen, expressed the serine protease granzyme B; (b) the lipid A induced the release of this protease, which was subsequently relocalized into tumor cells undergoing apoptosis; (c) phenotypically, the lipid A triggered a switch of Neutrophil/G-MDSC from cells expressing arginase-1 to cells expressing inducible nitric oxide synthase; (d) functionally, Neutrophil/G-MDSC cells were immunosuppressive, a function which was alleviated by lipid A. These findings support a role of a subset of Neutrophil/G-MDSC-expressing granzyme B in tumor regression induced by a lipid A, and propose a strategy in colon cancer cell therapy.

PO28

PROGNOSTIC VALUE OF SOLUBLE FASL AND TRAIL IN PATIENTS WITH BLADDER CANCER

Auteurs et adresses : Islem Ben Bahria-Sediki1,2,3, Carla Sampaio1,2, Amel Ben Ammar Gaied3,, Ali Bettaieb1,2,.

Auteur présentant le résumé : Islem Ben Bahria-Sediki

Abstract :
Activation of death receptor pathways in cancer cells triggers apoptosis. Soluble FasL (sFasL) and TRAIL (sTRAIL) can protect cells from apoptosis. Here, the significance of these soluble ligands in bladder cancer of Tunisian patients has been studied.
The levels of sFasL and sTRAIL in the serum of 64 patients and 72 healthy donors were determined by ELISA. Cytotoxicity was examined using the tetrazolium viability assay. Protein expression was analyzed by Western blot.
The mean serum level of sFasL was higher in patients than in normal donors, whereas no difference was seen between sTRAIL from patients and controls. sFasL was only higher than in sera of healthy donors where patients had superficial stage or low- and medium-grade cancer, or no recurrence. sTRAIL was significantly lower only in sera from patients with invasive and high-grade bladder carcinoma than in controls. The serum levels of sFasL and sTRAIL in patients with superficial non-invasive bladder tumors or low- and medium-grade cancers was higher than for invasive carcinomas and high-grade cancers.
The data suggest that elevated levels of bioactive sFasL and sTRAIL in the serum might be associated with a good prognosis in patients of the Tunisian population with bladder cancer.

PO29

ANTICANCER AGENTS: DOES A PHOSPHONIUM BEHAVE LIKE A PHOSPHINE GOLD(I) COMPLEX? LET A "SMART" PROBE ANSWER!

Auteurs et adresses : Lucile Dondaine,b Moussa Ali,a Anais Adolle,b Carla Sampaio,b Philippe Richard,a Franck Denat,a Ali Bettaieb,b Pierre Le Gendre,a Véronique Laurens,b Christine Goze,a Catherine Paul,b* and Ewen Bodioa*.

Auteur présentant le résumé : Lucile Dondaine

Abstract :
Gold phosphine complexes, such as auranofin, are known for decades for their use as antirheumatic agent. Clinical trials are now underway to validate their use as anti-cancer or anti-HIV treatment. However, their mechanism of action is still not clear, although many hypotheses have been advanced. One additional challenging question is to whether the gold phosphine complex is a prodrug or if the gold atom remains attached to the phosphine ligand. In this study we present two novel gold complexes which will be compared to auranofin and to their phosphonium analogue. The chosen ligand is a phosphine based smart probe, which fluorescence strongly depends on the presence of the gold atom. The in vitro biological action of the gold complexes and the phosphonium derivative were investigated, and preliminary in vivo study on healthy zebrafishes enabled us to evaluate their biodistribution and their toxicity. The different studies highlight the fact that the gold complexes seem to be stable and behave completely differently than phosphonium and auranofin in vitro as well as in vivo. Indeed, two photons microscopy experiments showed that the cellular targets of the gold complexes are not the same than those of the phosphonium analogue. Moreover, despite similar IC50 values in some cancer cell lines, gold complexes displayed a low in vivo toxicity, contrary to the phosphonium salt, and are therefore much more suitable for future in vivo investigation.

PO31

TUMOR PD-L1 EXPRESSION ACTS AS AN ADAPTATIVE IMMUNE RESISTANCE MECHANISM TO IMMUNOGENIC CELL DEATH

Auteurs et adresses : Magalie Dosset(1), Lucile Prost (1), Aurélie Roussey (1), François Ghiringhelli (1,2), Lionel Apetoh(1,2)
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Auteur présentant le résumé : Magalie Dosset

Abstract :
Despite the ability of immunogenic chemotherapies to induce immunogenic tumor cell death leading to CD8 T cell activation, these drugs often fail to induce complete tumor clearance. The mechanisms accounting for tumor resistance to chemotherapy-induced anticancer immunity remain unclear.
Here, we show that while combined treatment with drugs 5-Fluorouracil (5-FU) and Oxaliplatin (Ox) triggered immunogenic colon tumor death and transient CD8 T cell activation, these drugs concomitantly activates the programmed death-1 (PD-1) and PD-1 ligand (PD-L1) pathway, resulting in progressive CD8 T cell dysfunction and tumor escape.
Furthermore, we found that 5-FU/Ox, which induced tumor endoplasmic reticulum stress and persistent tumor antigen release, were also responsible for enhancement of PD-1 expression on CD8 T cells and tumor PD-L1 expression.
Finally, in two mouse colon tumor models, disruption of PD1/PD-L1 signaling in mice receiving 5-FU/Ox restored CD8 T cell function and led to complete long-lasting tumor clearance.
Our findings thus uncover what we believe to represent a novel resistance mechanism of cancer cells to immunogenic chemotherapies and provide impetus to combine chemotherapy with inhibitors of the PD-1/PD-L1 signaling pathway.

PO32

EVALUATION OF IMMUNO-ONCOLOGY RELATED TREATMENT IN SYNGENIC MOUSE MODELS

Auteurs et adresses : S. Maubant, M. Hillairet de Boisferon, F. Bichat, C. Mignard, X. Tizon, D. France, JF. Mirjolet
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Auteur présentant le résumé : Sylvie Maubant

Abstract :
Immunotherapy based on monoclonal antibodies targeting cancer cells is now developed as a valid approach to treat cancer. Modulation of novel immune checkpoints and other targets are highly promising approaches against cancer and many other diseases. They have the potential to activate the immune system and to establish an active defense against pathological conditions. In addition, antibodies, antibody fragments, and other biologics can also have a strong impact on the immune system which needs to be evaluated early on. In order to develop and accurately evaluate these immunology linked approaches, appropriate preclinical models with relevant immunological readouts are needed at different stages of therapy development. Ideally, methods should be available that allow predictive readouts in vivo and ex vivo.
A comprehensive panel of tools was constructed and validated aiming at evaluating the modulation of the immune system by new therapies.
In immunocompetent mice, immune cells were studied for the detection of their cell surface markers, induction of proliferative phenotype, antigen-specific T lymphocyte detection, secretion of soluble mediators using FACS phenotyping, cytometric bead assay (CBA) or Luminex multiplex technologies and ELISPOT. We report on a panel of syngenic tumor models (4T1, B16-F10, CT26, EMT6, AY27, LLC1, MBT-2 and Renca) our capacity to correlate subpopulation of immune infiltrating cells (as T cells, macrophages, NK cells, granulocytic and monocytic cells) and the therapeutic effects of critical antibodies directed against Cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death 1 membrane protein (PD-1).
In immunodeficient mice, the reconstitution of the mature human immune system with PBMC or naïve human immune system using hematopoietic stem cells was used to evaluate the modulation of those immune cells by therapies through human cytokine release and onset of graft versus host disease.
All of these tools were used in the context either of rodent syngenic models or humanized mouse models. In addition to their use in immune system modulating anti-cancer therapies, these humanized or syngenic models also have a potential application in many other therapeutic areas such as autoimmune diseases, inflammation, anti-infectives.

PO33

IDENTIFICATION D'UN NOUVEL ANTICORPS MONOCLONAL DIRIGE CONTRE LA MELANOTRANSFERRINE EXPRIMEE PAR LES CELLULES DE MELANOME

Auteurs et adresses : Jérôme Giustiniani, Yacine Merrouche:
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Armand Bensussan:
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INSERM U976 Hôpital Saint Louis, Paris.

Auteur présentant le résumé : Jérôme Giustiniani

Abstract :
Contexte: La mélanotransferrine, homologue de la transferrine impliquée dans le métabolisme du fer, est exprimée par les cellules de mélanome et les mélanocytes. Cette protéine est ancrée à la surface cellulaire par un motif glycosyl phosphatidyl inositol (GPI) et est libérée sous forme soluble par clivage protéolytique. Il a été démontré que la mélanotransferrine contribue à la tumorigénèse, à la migration des cellules cancéreuses et à leur invasion tissulaire. Cette protéine représente donc une cible thérapeutique potentielle dans le traitement des mélanomes.
Méthodes: Culture de lignées de mélanome. Modèle murin de xénogreffe de mélanome humain. Mesure de l'expression membranaire par cytométrie en flux et immunohistochimie. Identification du ligand par Western Blot.
Résultats: l'immunisation de souris avec une lignée humaine de mélanome nous a permis d'obtenir un nouvel anticorps monoclonal appelé CAMEL-1. Les résultats obtenus par cytométrie en flux et immunohistochimie montrent que cet anticorps a une réactivité sur les lignées de mélanome humain obtenues en laboratoire à partir de biopsies tumorales mais aussi sur des coupes congelées de métastases ganglionnaires ou de cellules de tumeurs primaires issues de xénogreffe indépendamment des passages sur les souris. Le ligand identifié par immunoprécipitation et Western Blot indiquent que l'anticorps CAMEL-1 reconnaît la mélanotransferrine exprimée par les mélanomes. Ce nouvel anticorps ouvre des opportunités dans le développement d'immunothérapie ciblant la mélanotransferrine.

PO34

EXPRESSION ET FONCTIONS DES DIFFERENTES ISOFORMES DE CD160 A LA SURFACE DES LYMPHOMES NK/T

Auteurs et adresses : Jérôme Giustiniani,Christian Garbar, Yacine Merrouche: laboratoire d'immunologie cellulaire et moléculaire et département de Bio-pathologies Institut Jean Godinot, Reims
Armand Bensussan: INSERM U976, Hopital Saint Louis, Paris

Auteur présentant le résumé : Jérôme Giustiniani

Abstract :
Expression et fonctions des différentes isoformes de CD160 à la surface des lymphomes NK/T
Context : Les lymphomes NK/T représentent environ 10 à 15% de l'ensemble des lymphomes non-Hodgkiniens. Leur nature agressive et leur mauvais pronostic ont attiré l'attention d'un besoin urgent de mieux connaître leur phénotype cellulaire associé à la fonction cytotoxique.
Un des récepteurs majeur de l'activité des cellules NK cytotoxiques est le récepteur CD160. Cette protéine, ancrée à la membrane par un motif GPI (Glycosyl Phosphatidyl Inositol) est présente, en autre, à la surface des cellules NK cytotoxiques. Une isoforme transmembranaire de CD160 (CD160-TM) a été identifiée et se trouve exprimée uniquement à la surface des cellules Natural Killer activées et de certaines lignées NK/T tumorales. Comme l'isoforme GPI, CD160-TM reconnait les molécules HLA classiques et non classiques. L'expression restreinte de CD160-TM aux seules cellules NK activées et potentiellement aux cellules NK/T tumoraux serait un argument important qui nous permettrait d'envisager d'utiliser ce récepteur transmembranaire dans une stratégie d'immuno-intervention.
Méthodes: Culture de différentes lignées de lymphome NK/T. Mesure de l'expression membranaire par cytométrie en flux. Détection des isoformes par Western Blot Détection par PCR des différents transcrits codant pour les isoformes de CD160 à partir d'échantillon de tumeurs.
Résultats : Nous avons montré que plusieurs lignées de lymphome NK/T expriment les transcrits codant pour les isoformes GPI et transmembranaire (TM) de CD160. Nous avons pu mettre en évidence le rôle activateur des fonctions cytotoxique et de sécrétion de cytokines de la forme CD160-GPI dans ces types cellulaires. Nous sommes entrain de développer les outils nécessaires, en particulier des anticorps spécifiques de la forme transmembranaire, pour étudier la fonction de CD160-TM dans ces mêmes cellules.

PO35

COMPARISON OF ABLATIVE IRRADIATION EFFECTS RELATIVE TO THOSE OBTAINED WITH NORMOFRACTIONATED IRRADIATION

Auteurs et adresses : Hélène Burckel, Elodie Josset, Delphine Antoni, Georges Noël
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Auteur présentant le résumé : Hélène Burckel

Abstract :
Purpose: Radiotherapy, used alone or in combination with surgery and/or chemotherapy, represents today 50 to 70% of anticancer treatments. Innovative techniques in radiotherapy such as intensity modulated radiation therapy (IMRT) and stereotactic radiotherapy allowed improving local control and shielding healthy critical organ surrounding the tumor. The protocols of radiation are based on clinical experience and radiobiological models. The linear-quadratic (LQ) model allows the prediction of therapeutic response and calculation of equivalent doses. However, some clinical trials have demonstrated the inability of this model to predict therapeutic response depending on the mode of delivery of the dose. Thus, a better understanding of the biology of ablative irradiation dose compared to standard normofractionated ones would avoid estimation from radiobiological models of conventional radiotherapy and permit to adapt the mathematical model.
Material and methods: Four human cancer cell lines were studied, including especially a glioblastoma cell line and a hepatocellular carcinoma cell line. Two irradiation schedules were compared: ablative irradiation doses of 10 and 20 Gy delivered in a unique fraction versus normofractionated doses of 5 or 10 fractions of 2 Gy (one fraction per day, 5 days a week). Some clonogenic survival assays, cell cycle distribution evaluations, cell death quantification (apoptosis, autophagy and necrosis) and radio-inducted DNA damages (double-strand breaks) analysis were performed.
Results: Two groups of response were observed in clonogenic survival with a lower surviving fraction with the ablative irradiation pattern. The cell cycle distribution differs relative to the irradiation schedule with a predominance of cells in G2/M phase for ablative doses. The rates of cells in apoptosis, autophagy and necrosis are fluctuating depending on the irradiation pattern and the evaluation time. 24h after irradiation, the remaining H2AX foci numbers, focusing the non-repaired DNA double-strand breaks is relatively low whatever the irradiation dose and the fractionation.
Conclusions: Some distinct differences have been obtained according to both irradiation patterns analyzed. It is probable that the irradiation-induced cell death occurs by distinct mechanisms depending on the irradiation schedules. Further investigations are required to improve the knowledge of the radiobiology of ablative irradiation in order to predict tumoral response and to adapt treatments.

PO36

PRE-CLINICAL DEVELOPMENT OF A DOCETAXEL NANOCARRIER TO ENHANCE PROSTATE CANCER RADIOSENSITIVITY

Auteurs et adresses : C. Mirjolet1, J. Boudon2, R. Boidot1, S. Chevrier1, C.Dalban1, A. Loiseau2, B. Collin1, A. Oudot1, T. Gautier2, N. Millot2 and G. Créhange1
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Auteur présentant le résumé : Céline Mirjolet

Abstract :
From 30% to 50% of high risk prostate cancer patients who undergo radiation therapy (RT) will have a biochemical failure. Combining chemotherapy, such as Docetaxel (DXL), with RT can enhance its efficiency. Multidrug resistance mechanisms often limit drug efficacy by decreasing tumor cell intracellular concentration of drugs. There is interest to develop nanocarrier of DXL to maintain drug inside cancer cells by improving its efficacy. The purpose of this study was to develop a titanate oxyde-nanotube (TiONt)-DXL nanocarrier (nanohybrid) and to evaluate its in vivo biodistribution as well as its radiosensitizing efficacy in association with RT on a hormone-independent prostate cancer model.
DXL molecules were grafted on TiONts using PEG-3000 molecules to generate the nanohybrid. In vitro cytotoxic activity of the nanohybrid was evaluated on PC-3 cell line using MTS assay. After intratumoral injection, biodistribution analysis was performed by SPEC-CT imaging of mice bearing subcutaneous PC-3 human prostate tumors. To evaluate the benefit of nanohybrid and RT association, tumors were irradiated using 3 fractions of 4Gy administrated after the injection of nanohybrids. Nine groups of 7 mice were used to evaluate nanohybrid and RT association efficacy : untreated, control with buffer IT injection, +/- RT, free DXL, +/- RT, TiONt +/- RT and TiONt-DXL +/- RT. Mice behavior, health status and tumor volume were monitored twice a week until tumor growth recovery.
Biodistribution kinetics showed that more than 70% of nanohybrids were localized into the tumor 96 hours after injection. Mice receiving nanohybrid-RT exhibited a significant tumor growth delay to reach a volume of 1,000mm3 compared to mice receiving free DXL-RT: 73.7 days (median, [58.9-89]) vs 56 days (median, [31.5-74]) (p=0.0127).
TiONt-DXL improves RT efficacy compared to free DXL. These results suggest that local control might be enhanced by TiONt-DXL. TiONt-DXL can be injected as a radiosensitizer in men harboring high risk localized prostate cancer with needles during prostate brachytherapy procedure.
This work was supported by the "Ligue Contre le Cancer du Grand Est" (Comités Doubs and Côte d'Or).

PO37

TOXICITE DES IONS CARBONE EN COMPARAISON DES RAYONS X DANS DES FIBROBLASTES DE PEAU

Auteurs et adresses : Carine Laurent1, Alexandre Leduc1, Ivannah Pottier2,3,4, Virginie Prévost2,4,5, Stéphanie Lagadu2,3,4, Jean-Louis Lefaix6 & François Sichel2,3,4
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Auteur présentant le résumé : Carine Laurent

Abstract :
Objectifs : Des travaux ont rapporté l'apparition de lésions cutanées après hadronthérapie par ions carbone (i-C). Cette étude cherche à évaluer le stress oxydatif et les voies impliquées dans des fibroblastes de derme humains sains après irradiation aux i-C vs. rayons X (RX).
Matériels et Méthodes : Après étude de la clonogénicité, le stress oxydatif a été quantifié (lésions des macromolécules biologiques, défenses antioxydantes) et la sécrétion de cytokines proinflammatoires évaluée. La transcription de 168 gènes a été étudiée. Sur les gènes sélectionnés, un traitement antioxydant a été testé.
Résultats : L'EBR à D0 des i-C vs. RX est de 4,8. Les lésions primaires de l'ADN sont moindres après i-C vs. RX, avant une augmentation tardive observée uniquement après i-C. Les produits d'oxydation des lipides et des protéines sont augmentés uniquement après i-C. Les activités des enzymes anti-oxydantes sont diminuées après i-C vs. RX. Le ratio GSH/GSSG, inchangé après RX, est diminué immédiatement après i-C avant une augmentation à 7 jours. L'augmentation de la sécrétion de 8-oxo-dG est accentuée après i-C aux temps tardifs. La sécrétion d'IL-6 est diminuée immédiatement après RX ou i-C avant une augmentation à 14 jours. Après i-C et vs. RX, la transcription des gènes des défenses anti-oxydantes, des arrêts dans le cycle cellulaire et la sénescence, de l'inflammation et de l'apoptose était diminuée. Au contraire, la transcription des gènes de réparation de l'ADN et du choc thermique était augmentée. Sur les 168 gènes étudiés, 22 ont été sélectionnés pour leur forte régulation. Un traitement antioxydant a permis d'augmenter la transcription de gènes qui était inhibée par les i-C et de diminuer la transcription de gènes qui était activée par les i-C.
Conclusions : Les i-C sont particulièrement néfastes pour les fibroblastes sains puisqu'ils entraînent une diminution de la transcription de gènes impliqués dans les arrêts dans le cycle & la sénescence, une diminution de la capacité antioxydante, et une augmentation des lésions des macromolécules biologiques. Le traitement antioxydant a confirmé l'importance du stress oxydatif induit par les i-C.

PO40

DEVELOPMENT OF NEW [18F]-FLUORO CARBOHYDRATE-BASED PROSTHETIC GROUPS AND THEIR CONJUGATION TO PEPTIDES VIA CLICK CHEMISTRY

Auteurs et adresses : Charlotte Collet,Fatiha Maskali,Françoise Chrétien, Sylvain Poussier, Gilles Karchera, Pierre-Yves Marie,Yves Chapleura, Sandrine Lamandé-Langle
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Auteur présentant le résumé : Charlotte Collet

Abstract :
This work describes the synthesis and 18F-labeling of new [18F]fluoro carbohydrate-based prosthetic groups equipped with an azido arm that are able to participate in copper(I)-catalyzed cycloadditions for 18F labeling of biomolecules under mild conditions. The synthesis and radiolabeling in high radiochemical yields (up to 67 ± 6%) of these differents prosthetic groups were presented. The flexibility of the azido arm introduced on the carbohydrate moietie allows efficient click reactions with different alkyne functionalized peptides such as gluthation or RGD derivatives to prepare glycopeptide. The synthesis and radiosynthesis of this corresponding glycopeptides proceed in high radiochemical yields of up to 75% to provide 18F-labeled glycopeptides in a fully automated process. The addition of a sugar moitie on peptides should enhance the bioavailability, pharmacokinetic and in vivo clearance properties of these glycopeptides compare to the corresponding peptide for their use as radiotracers in PET scan imaging.

PO42

NOUVELLES TECHNOLOGIES DE BIOPHOTONIQUE CELLULAIRE APPLIQUEES A L'ONCOLOGIE

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Auteur présentant le résumé : A. El-Bakri

Abstract :
INTRODUCTION ET OBJECTIFS :
Les techniques de spectroscopie vibrationnelle peuvent être utilisées pour la détection des changements qui se produisent au niveau cellulaire et moléculaire au cours de la carcinogénèse des tissus. Le potentiel de leur utilisation en oncologie est encore à ses balbutiements et l'augmentation de l'utilité de cette nouvelle technologie va transformer le diagnostic et le pronostic tissulaire non invasifs.
MATÉRIELS ET MÉTHODES :
Un état de l'art a été réalisé sur les applications de la biospectroscopie en cancérologie dans la vessie, le rein, la prostate, le colon, les tumeurs cérébrales et le mélanome. Une revue systématique a été conduite dans Pubmed et l'expérience des deux seuls centres Français de biophotonique a été également rapportée. 30 articles ont été sélectionnés.
RÉSULTATS :
Les technologies de biophotonique cellulaire notamment la spectroscopie Raman et la microspectroscopie infrarouge à transformée de Fourier sont utilisées pour interroger les tissus biologiques. Leur utilisation potentielle en oncologie a donné des résultats encourageants dans le diagnostic in vitro des cancers de la vessie, prostate, colon, mélanome. Dans le carcinome rénal la fiabilité diagnostique peut aller jusqu'à 85%. Ces techniques peuvent être effectuées sur des tissus en paraffine type Tissue Microarray (TMA) en utilisant un algorithme de déparaffinage numérique validé. Une empreinte spectrale cellulaire spécifique est produite et peut aider à l'élaboration de nouveaux biomarqueurs moléculaires mais également à mesurer la réponse thérapeutique aux anti-cancéreux. Ces techniques peuvent également être utilisées in vivo pour identifier les marges peropératoires en neurochirurgie ou lors de la néphrectomie partielle et la prostatectomie radicale. L'avenir verra le développement potentiel de sondes à fibres optiques pour les intégrer dans les cathéters, endoscopes et laparoscopes.
CONCLUSION :
La spectroscopie est un outil intéressant pour le diagnostic en temps réel et l'évaluation in vitro ou in vivo des tissus cancéreux et permet d'établir une signature optique biomoléculaire spécifique.
Les applications potentielles de cette imagerie du vivant annoncent un nouvel avenir en chirurgie des cancers notamment dans la différenciation des pathologies malignes ou précancéreuses par rapport à des tumeurs bénignes ou en cas de doute opératoire concernant les marges.
MOTS-CLÉS : Spectroscopie Raman ; urologie ; cancérologie ; microspectroscopie ; biophotonique ; spectroscopie infrarouge à transformée de Fourier ; chirurgie oncologique ; spectroscopie vibrationnelle ; marges

PO43

INFRARED SPECTRAL DIAGNOSIS FOR PREDICTIVE CANCER MEDICINE: APPLICATION TO THE EARLY DIAGNOSIS AND PROGNOSIS OF PRE-INVASIVE BRONCHIAL INTRAEPITHELIAL LESIONS

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Auteur présentant le résumé : Vincent Gaydou

Abstract :
This study concerns the development of a new imaging diagnostic tool for bronchial cancer. This malignancy is a wide-spread cancer in the world, with an increasing incidence. Our objective is to assess the potential of infrared (IR) spectroscopy in the early diagnosis of the bronchial cancer. Our approach is divided into two steps: the first issue aims at highlighting specific spectroscopic markers of the bronchial cancer permitting to detect unambiguously the presence of cancer cells. The second step relies on establishing a chemometric model able to estimate the tumor invasivity of these malignant cells
For our approach, we used 4 different bronchial cellular lines displaying different in vitro invasive properties assessed by a modified Boyden chamber assay : the non invasive cell line 16HBE used as a control, the moderate invasive cell line Beas2B and the two highly invasive cell lines BZR and BZRT33. Four samples of each cell line were cultured and embedded in paraffin wax. Slices of 10 µm were prepared on CaF2 windows suitable for IR measurements. The infrared acquisitions were performed with an imager (Bruker, Germany) equipped with a Focal Plane Array detector allowing to image large tissue areas with a spatial resolution of a few micrometers.
The IR spectra were processed using chemometric and statistical multivariate treatments. A first stage permitted to correct spectral interferences originated from the paraffin and optical effects. Then, supervised models were established by means of PLS-DA (Partial Least Square Discriminant Analysis) method. A first model, based on sparse-PLS method, was optimized to highlight spectroscopic markers specific of the normal and cancerous states; this model has proved effective in terms of sensibility (96%) and specificity (90%). Based on these promising results, the study will be pursued by the analysis of tissue samples from biopsies and tumor samples. Subsequently, the next investigation will rely on the construction of a second model to model and quantify the aggressiveness level of the cell lines.

PO46

ASSESSMENT OF EXTRAMEDULLARY HEMATOPOIESIS (EMH) BY HYBRID IMAGING IN PATIENTS WITH PRIMARY MYELOFIBROSIS (PMF)

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Auteur présentant le résumé : Mario Ojeda Uribe

Abstract :
The long-term (along the whole life) dynamic relationship between the skeleton and the bone marrow (BM) hematopoietic system opens the way to the development of diseases that can be derived from signal-transduction abnormalities in hematopoietic stem cells (HSC) (genetically driven by some somatic mutations like those involving the genes JAK2, CALR or MPL) and/or from structural deficiencies in the BM micro-environment. At least 2 hematopoietic BM niches have been described: a) the endosteal (or osteoblastic) niche where the HSC are in close contact with the surfaces of osteoblasts and b) the vascular niche where the HSC are located close the endothelial cells, the MSC and the pericytes surrounding the sinusoidal structures of the BM. This dynamic relationship between the HSC, their niches and the bone structure in particular the trabecular bone where the normal hematopoiesis is settled is severely disrupted in some myeloproliferative neoplasms (MPN) as PMF.
PMF is a kind of MPN characterized among other features by the development of an EMH. Several tracers can be used in nuclear medicine for diagnosing EMH. Colloids labelled with Technetium 99m (99mTc) show the reticulo-endothelial system which reflects the richness of some components of the medullary micro-environment. Indium 111-transferrin can reflect the medullary cell activity
To assess the interest of non-invasive nuclear medicine procedures with the use of 99mTc and In111 coupled to a CT-Scan to visualize the extent and intensity of the EMH in PMF.
To assess the usefulness of this hybrid imaging technique to measure the response to anti-JAK2 drugs in PMF.
We performed hybrid imaging procedures in 4 untreated patients with PMF and in 1 patient with essential thrombocytemia (ET). Two tracers were employed: a) 99mTc: Whole body and SPECT acquisitions were made with a dual head gamma camera (Hawkeye, GE). The whole body acquisitions were made 1 hour and 6 hours after IV administration of 99mTc colloids, with the following acquisition parameters: sweep speed 12 cm/min, low energy collimator, A SPECT/CT was also performed with multiplanar reconstructions medium resolution ; b) Indium-111 (111In) transferrin scintigraphy (Indium chloride labelled autologous plasma transferrin) with SPECT/CT. Scintigraphy image acquisitions were made 48 and 72 hours after injection of 111In-transferrin.
In the PMF patients we observed a low intensity fixation of both tracers in the axial skeleton and conversely a hyperfixation at the level of the limbs and the spleen. Interestingly in the patient with ET who was developing a secondary myelofibrosis the hyperfixation was seen only in the spleen. No other EMH localizations were observed. The use of this hybrid imaging allowed a good evaluation of the extent and intensity of the EMH in patients with PMF. Its use as a tool to assess the response to anti-JAK2 drugs needs further evaluation.

PO47

PHASE 0 CLINICAL TRIALS: A FRENCH PATIENT'S POINT OF VIEW

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Auteur présentant le résumé : Nicolas Isambert

Abstract :
Phase 0 clinical trials are a new phase of the development of drug linking preclinical research and clinical studies. During these first-in-man studies, only a few small doses of a new drug are delivered on a very limited number of patients. They aim at verifying whether the drug reaches its target in the tumor and then help researchers to control whether the mechanisms of action described in preclinical model are the same as in human. So, the risk of toxicity is limited by the use of small doses of drug but one major disadvantage is the need for additional blood samples, imaging tests and especially biopsies. Then, no therapeutic or diagnostic intent is expected for patients. However, this process is crucial, it may help to avoid the delay and expense of finding out in later phases when the drug doesn't act as expected. Phase 0 studies are not yet being used widely and several authors emphasized on the role of patient in the implementation of these trials. We aimed at evaluating the knowledge and acceptability of phase 0 studies among a cohort of French people.
A short questionnaire was sent to the members of the French committee for clinical research by the "Ligue Nationale Contre le Cancer". This committee consists of 48 individuals: either patients with cancer or relatives of patients with cancer. Their mission is to proofread and give notice on projects submitted by clinical trial sponsors.
27 of the 48 members (56%) sent us back the questionnaire. Eleven (40%) were able to point out the correct definition of phase 0 studies. The majority of responders (n=20, 73%) had some concerns about the burden for the patient included in this type of study and would probably not give a favorable advice when exanimating a phase 0 project. Nevertheless, half of the responders were opposed to enrollment of healthy volunteers, whereas only patients with cancer may be included in this type of study.
In conclusion, before implementation of phase 0 studies in France, many points should be clarified. Patients and their close relatives should be included in the afterthought and communication about phase 0 studies both towards clinicians and patients.

PO48

INVESTIGATING THE INFLUENCE OF LRP-1 SILENCING BY ATOMIC FORCE MICROSCOPY REVEALS A PROMISING NON-INVASIVE TOOL TO ESTABLISH A QUANTITATIVE LINK BETWEEN MECHANICAL, ADHESIVE CELLULAR PROFILES AND THE EVOLUTION OF A CELLULAR DISORDER SUCH AS METASTASIS

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Auteur présentant le résumé : Lionel Chièze

Abstract :
The low-density lipoprotein receptor-related protein-1 (LRP-1) is an endocytic receptor mediating the extracellular amounts of various matrix metalloproteinases (MMPs) involved in the dissemination of cancer cells. LRP-1-dependant endocytosis has been widely studied as a potential target against cell invasion, and was found to have prognostic value. Recent datas from our laboratory have provided evidence that cell invasion is decreased by LRP-1 silencing, despite the predicted increase in pericellular proteolytic activities. Various in vitro techniques are traditionally used to assess the metastatic potential of cells, based on cell morphology, migration behaviour, invasive capabilities and the expression of specific molecules or genes. Since the cytoskeleton is the main contributor of cell stiffness and that metastasis is marked by profound alterations in cytoskeleton dynamics, atomic force microscopy (AFM) has been one of the techniques which contributed to the growing interest towards mechanical properties of cancerous cells and how they are associated with metastatic potential. In this work, we knocked down LRP-1 in the human thyroid carcinoma cell line FTC-133 through a shRNA strategy and we used AFM in order to establish a specific mechanical characterization of LRP-1 silenced cell phenotype. Moreover, by using colloidal probes functionalized with gelatin, we have measured the adhesive strength during the initiation of cell adhesion.
Results show that LRP-1 silenced cells are characterized i) by a faster cell adhesion to gelatin resulting to cell spreading and ii) by an increase of cell stiffness associated with the numerous stress fibers. Our results demonstrate that the non-metastatic phenotype of LRP-1 silenced cells is similar to the phenotype of others well described non metastatic cell lines. AFM appears as a promising non-invasive tool to establish a quantitative link between mechanical or adhesive cellular profiles and the evolution of a cellular disorder such as metastasis. Further investigations involving tip functionalization with antibodies directed against integrins subunits, could allow us to extend our knowledge of the effect of LRP-1 silencing on mechano-transduction itself at the mechanical level.

PO50

NUCLEAR IMAGING STUDY OF THE EFFECTS OF DEBIO 1143, A NEW ORAL SMAC MIMETIC INDUCING APOPTOSIS IN A TRIPLE-NEGATIVE BREAST CANCER MODEL

Auteurs et adresses : Oudot A, Raguin O, Bauché L, Bichat F, Vaslin A, Maby-El Hajjami H, Zanna C, Vuagniaux G, Fumoleau P, Brunotte F and Collin B.

Auteur présentant le résumé : Bertrand Collin

Abstract :
Introduction: High expression levels of inhibitor of apoptosis (IAP) proteins have been encountered in various human cancers. Since IAPs are involved in tumorigenesis, cancer progression, treatment resistance and poor prognosis, they represent an attractive target for therapeutic intervention. Debio 1143 (formerly named AT-406), a new potent orally-available monovalent SMAC mimetic, targets multiple IAP members and is currently in clinical trials for cancer treatment. In this study, nuclear imaging was used to evaluate the effects of Debio 1143 on tumor cell death by single photon emission computed tomography/computed tomography (SPECT), and on metabolism by positron emission tomography (PET) in the triple negative breast cancer (TNBC) cell line MDA-MB-231. Material and Methods: The effects of Debio 1143 on caspase-3 activation (FACS), apoptosis-induced membrane changes (FACS and radioactive detection of annexin V binding), and cell proliferation (MTS assay) were evaluated in MDA-MB-231 cells. In vivo pharmaco-imaging experiments were performed in CB17 SCID mice bearing subcutaneous MDA-MB-231 TNBC tumors. 99mTc-annexin V SPECT/CT imaging was performed 6 and 24 hours after single-dose treatment with the vehicle, Debio 1143 (100 mg/kg per os) or paclitaxel (7.5 mg/kg intravenously), and was completed by gamma counting of organs. Tumor metabolism was evaluated by 18F-FDG (fluorodeoxyglucose) PET/CT while animals received repeated administrations of vehicle, Debio 1143 (100 mg/kg per os) or paclitaxel (7.5 mg/kg intravenously). Results: In MDA-MB-231 cells, Debio 1143 as a single agent was highly effective in inducing apoptosis. This was reflected by a dose-dependent activation of caspase-3, an increase of annexin V cell binding, and a cytotoxic activity with a mean IC50 of 224 nM. In MDA-MB-231 tumor-bearing mice, Debio 1143 showed an effect on 99mTc-annexin V tumor binding with increased tumor SPECT signal and gamma counting results 6 hours after oral administration, while paclitaxel maximum effects were detected at 24 hours post-treatment. During repeated administration, oral Debio 1143 inhibited tumor growth, which was associated with a decreased tumor 18F-FDG uptake when measured during treatment (after 1 and 2 weeks of treatment). Effects on tumor growth and 18F-FDG uptake were still present 1 week after the end of the treatment. Conclusion: This nuclear imaging study showed that Debio 1143 induces apoptosis both in vitro and in vivo in a TNBC model. Moreover, 18F-FDG PET imaging demonstrated potent effects of Debio 1143 over time, suggesting that this imaging technique may be useful in the clinical setting. In conclusion, Debio 1143 treatment represents a promising approach to managing TNBC, still a highly unmet medical need.

PO51

FOLLICULAR HELPER T CELLS IN OVARIAN CANCER

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Auteur présentant le résumé : Delphine Hudry

Abstract :
Objectives: Immune cells infiltrating the tumour microenvironment control tumour progression, as illustrated by reports indicating that tumour infiltration composition is associated with vital prognosis of cancer patients. Follicular helper T (T_{FH}) cells are a CD4+ lymphocyte subpopulation described in 2000. These cells are essential for B-cell maturation and *de facto* in antibody production. Although the discovery of T_{FH} dates back to almost fifteen years, their role in human disease and more particularly in cancer progression still remains controversial. Recently, T_{FH} cells were found to infiltrate solid-organ tumors, and their presence was associated with increased survival in breast cancer for example. To advanced ovarian cancer, it has been shown that intratumoral T cells were correlated with improved clinical outcome. Our study was designed to analyze CD4+ T cells and especially T_{FH} cells in advanced ovarian cancer.
Methods: The patients included in the study had ovarian cancer supported by first surgery or neoadjuvant chemotherapy. Peripheral blood, carcinomatosis, ascites were analyzed. To identify T_{FH} cells by flow cytometry, we use specific profile: CXCR5 and PD-1. Women after surgery for benign disease, prolapse, prophylactic oophorectomy, allowed comparing the results, with the taking of peripheral blood and peritoneum.
Results: The amount of circulating T_{FH} cells seems higher in patients compared to controls. The distribution of T cells in ascite appears to correspond to carcinomatosis or primary ovarian tumor. The amount of T_{FH} in carcinomatosis is greater than in the "healthy" peritoneum.
Perspectives: We aim to determine that T_{FH} part between healthy volunteers and patients is different. Moreover, to different patients, TFH part is variable. T_{FH} cells could become a prognosis factor in advanced ovarian cancer.

PO52

DEGLYCOSYLATED BLEOMYCIN, WHILE KEEPING BLEOMYCIN'S ANTITUMOR ACTIVITY, LACKS ITS PULMONARY TOXICITY

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Auteur présentant le résumé : Guillaume Wettstein

Abstract :

Introduction : The therapeutic efficacy of the chemo-agent bleomycin (BLM) is restrained because of its lung toxicity with pulmonary fibrosis (PF) being the most devastating form. The deglycosylated form of BLM (deglyco-BLM) is obtained by chemical synthesis. DBLM and BLM are both able to induce tumoral cell apoptosis *in vitro*. We assessed DBLM anticancerous activity and lung toxicity *in vivo*.

Methods : BLM and deglyco-BLM were administred intraperitoneally in rodent models of tumor (including human Hodgkin lymphoma xenograft and syngeneic melanoma model). Lung toxicity was assessed *in vivo* by intra-tracheal administration of BLM, deglyco-BLM or NaCl in C57BL/6 mice and *in vitro* on epithelial A549 cells.

Results : We demonstrate *in vivo* in different rodent cancer models that intra-peritoneal deglyco-BLM is as effective as BLM in inducing tumour regression. However, while this BLM-induced anti-tumour effect was accompanied by a loss in body weight, deglyco-BLM did not affect body weight. When administered intratrachealy to C57BL/6 mice, deglyco-BLM did not induced collagen accumulation (Histomorphometry) and PF (Masson Trichrome) compared to BLM-injected mice lung. Interestingly, while both molecules were able to significantly induce lung epithelial cells apoptosis after intra-tracheal administration, deglyco-BLM lost the ability to induce the production of ROS, transforming growth factor-β1 and other pro-fibrotic and inflammatory cytokines in the lungs of mice.

Conclusion : This study highlights the antitumorigenic properties of deglyco-BLM, the modified form of BLM. deglyco-BLM does not induce PF in rodent. We propose that deglyco-BLM should be considered a clinical less-toxic alternative to BLM in cancer therapy.

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