496 Inhibition of Akt pathway restores the sensitivity to cetuximab in a head and neck squamous cancer cell line

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The Epidermal Growth Factor Receptor (EGFR) is a central regulator of cell proliferation in human cancers and is frequently overexpressed in many types of tumours. Cetuximab is a monoclonal antibody designed to block the EGFR activation, to induce the internalization of the receptor and to reduce the signaling pathway. However many tumours exert resistance to EGFR inhibitors. Accumulating evidence indicates that the response of cancer cells to cetuximab is a complex process that can be affected by multiple resistance mechanisms.
The aim of this study was to investigate the mechanisms of resistance to cetuximab treatment *in vitro.* We investigated the EGFR pathway, the expression of HER3 and HER ligands, the EGFR internalization after cetuximab treatment, alone or combined, and we compared results in CAL33 (HNSCC) and A431 (epidermoïd cancer) cell lines which present different sensitivities to cetuximab.
Cetuximab induced a high growth inhibition and a high inhibition of ERK and AKT phosphorylation in A431. By contrast, cetuximab induced a lower growth inhibition, an ERK phosphorylation inhibition but any inhibition of the AKT pathway. The cetuximab sensitivity of these cell lines was thus different and the difference would be based on the AKT pathway. To verify this hypothesis, we used an EGFR tyrosine kinase inhibitor and several AKT inhibitors. Results have shown that the AKT pathway can be inhibited in CAL33 cell line by an EGFR tyrosine kinase inhibitor and an AKT inhibitor. While the cetuximab induced a strong growth inhibition in A431, this growth inhibition was weakly increased by the combination. In contrast, cetuximab induced a weak growth inhibition in CAL33 while the growth inhibition was much stronger with the combination than in A431. We investigated the EGFR internalization role in these models. Preliminary results showed significant differences between these two cell lines.
In conclusion, this study had shown some explanations to the limited efficacy to cetuximab in CAL33. Firstly, we showed a persistent activation of AKT in CAL33 which might prevent the antitumour effect of cetuximab. Secondly, we have shown that the EGFR internalization and signal transduction mediation might contribute to the response to cetuximab in CAL33 and A431. The AKT pathway appears as a central element in the cetuximab sensitivity in these models and the combination of cetuximab with an AKT inhibitor could be a good therapeutic option in HNSCC.

497 Liver microenvironment stimulates aggressiveness of colorectal tumour cells more efficiently than matched primary tumour microenvironment – Hepatic CAFs induce ERK-mediated modification of cell morphology

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Background: Carcinoma-associated fibroblasts (CAFs), the more abundant cells in tumour stroma, are important contributors in determining the tumour's fate by establishing paracrine communication and secreting factors that enhance tumour progression. This study aimed to assess the influence of liver microenvironment on the progression of colorectal carcinoma.
Material and Methods: Conditioned media (CM) from matched normal colonic fibroblasts (NCF), CAFs from primary tumour (CAFpt) or liver metastasis (CAFlm) was obtained from a patient with colorectal cancer. We performed proliferation, colony formation, migration and invasion assays in DLD-1 and SW480 cells. Microarray and GSEA-analysis were performed in DLD-1 cells cultured in different CM. Proteomic evaluation of soluble factors secreted by fibroblasts was also carried out.
Results: Whereas CM from CAFpt and NCF increased proliferation of DLD-1 and SW480 cells, liver CAFs (CAFlm) induced an inhibition in relation to control DMEMF12. CM from hepatic CAFs (CM CAFlm) encouraged a more aggressive phenotype in colorectal cells determined by stimulation of motility, migration and invasiveness and resulted in cells differentiating towards a "neuronal-like" morphology, showing an extended formation of invadopodia and lamellipodia. Such changes correlate with a sustained activation of the ERK-pathway in DLD-1 cells. Differential transcriptomic profile of DLD-1 cells treated with conditioned media from CAFlm depicted overexpressed genes like ARF6, ACTR2 and RHOB that correlate with such morphology and in addition, associated with a prognostic signature of colorectal carcinoma in a GSEA-analysis. Proteomic analysis showed TIMP-1, SPARC, PAI-1 and collagen-a-1 as the more relevant exclusively detected in CM CAFlm. Validation in an independent set of 16 NCF, 16 CAFpt (10 paired), 3 hepatic stellate cells and 6 CAFlm (2 paired) and in 40 matched normal colonic mucosa/primary tumour/liver metastasis specimens showed overexpression of PAI-1, TIMP-1 and collagen-a-1 but not SPARC in liver microenvironment.
Conclusions: We describe that under the same genetic background, liver microenvironment provides more favourable conditions for colorectal cells to become more aggressive.

498 Telomere length as indicator of transposon silencing and cell genome stability

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Background: We discovered recently that 5′-CG-3′ and 5′-CNG-3′ sites are found in interfering RNAs (RNAi) sequences more often than they should be found in random DNA sequence. Therefore we hypothesized that binding of complementary DNA sequence by RNAi leads to recruiting of DNA methyltransferases that methylate *de novo* cytosine in 5′-CG-3′ and 5′-CNG-3′ sites of DNA, which appeared to be bound with similar sites in the RNAi sequence. As microRNA genes are the transposable element derivates, we supposed that mechanism of RNAi-directed DNA methylation appeared in the evolution with the purpose of silencing of the mobile genomic elements. Specific set of RNAi expressing in the stem cells can restore initial profile of their epigenetic markers through this mechanism, thus minimal level of the transposons activity is achieved and immortal status of stem cells is supported for ever.
Material and Methods: Prediction of microRNA targets within transcripts of stage-specific genes using TargetScan software (http://www.targetscan.org/).
Results: Transcripts of great number of stage-specific genes are the targets of the cell microRNAs. Therefore cell differentiation, starting with the earliest stages, must be accompanied with repression of some microRNA genes, otherwise these microRNAs would prevent expression of the stage-specific genes.
Conclusions: Differentiating cells can lose slowly the repressive chromatin markers because of the silencing of microRNAs genes that are essential for renewal of these markers through the RNAi-directed DNA methylation. This will excite the derepression of silent transposons with time, subsequent increase of level of DNA damages induced by them and following activation of cell DNA repair system including mechanisms based on homologous recombination. In our opinion, these mechanisms cause not only the DNA repair, but also illegitimate recombinations in telomere caps, since they are pre-recombination structures. As a result, the T-loops converse into rings and, accordingly, telomeres are shortened for the length of the lost circled DNA (50–500 bp) that exceeds few times DNA loss over the end-replication problem (3−5 bp).
This process can cause exhaustion of telomeres in cells, in which the activation of recombination process becomes apparent. Usually, telomere shortening has to correlate with duration and intensity of repair system activity, i.e. with severity of DNA damage and, consequently, probability of cell transformation. Thus, proliferative ability of these compromised cells is restricted. The telomere length is integral indicator of genomic stability in normal cells.
Apparently, large quantity of organism cells reaches with age the threshold of illegitimate activation of silent mobile genomic elements. Following apoptosis of most of these cells causes the ageing as biological phenomenon, while the transposon-mediated transformation of their part determines correlation between ageing and cancer appearance.

499 Prox1 expression in liver metastases from colon carcinoma

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Background: Scattered data reported the Prox1 involvement in dysplastic transformation of colonic adenomas on murine experimentral model and its role in progression of colonic adenocarcinomas. No or rare Prox1 positive cells were found in normal colonic mucosa especially in basal cells. The aim of this work was to identify presence and characteristic features of Prox1 positive cells in liver metastasis from colon carcinomas in human specimens.
Material and Methods: Our study included 12 liver biopsies from patients previously diagnosed with colon carcinoma (7 metastases from well differentiated adenocarcinomas, 3 from moderate differentiated carcinoma and 2 from poorly differentiated colon carcinoma). Resected liver specimens obtained by open surgery were fixed in 10% bufferred formalin and parrafin embedded. Immunohistochemistry was performed by using Prox-1 polyclonal antibody followed by incubation with ADVANCE/HRP system and 3,3 diaminobenzidine as chromogen.