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Gefitinib in liver cancer: could laminin-5 expression be a good predictive factor?

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Background: Hepatocellular carcinoma (HCC) is one of the most lethal malignancies and no current adjuvant or palliative treatment modalities have been conclusively shown to prolong survival. In the USA, a Phase II trial evaluating the efficacy of gefitinib (Iressa®), an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), in patients with advanced unresectable HCC is ongoing. In this report, we provide preclinical evidence of gefitinib activity using an *in vitro* HCC model. Moreover, the extracellular matrix can promote chemotherapeutic resistance in HCC cells (the mechanism is unknown). As laminin-5 is expressed only in hepatocarcinoma tissues, we have evaluated whether this component of the extracellular matrix can modulate gefitinib activity in our HCC model and the steps involved in this mechanism.
Materials and Methods: The study was carried out using two liver cancer cell lines; HLF and Alexander. Initially, both were evaluated for any drug-dependent modulation of the expression and activity of receptors (EGFR, Neu and KDR) and signal transduction pathway effectors (Akt, Erk1/2, etc), using immunoprecipitation and Western blot analyses. In order to evaluate sensitivity to gefitinib, cell growth inhibition was measured using a colorimetric (microtiter [MTT]) assay as a function of time and concentration, and the IC_{50} was obtained (CalcuSyn software, Biosoft). Both cell lines were then exposed to gefitinib (IC_{50}) and laminin-5 (1 μg/mL) alone or in combination in a colorimetric (MTT) assay to determine whether laminin-5 could reduce gefitinib activity. All *in vitro* experiments were performed in triplicate and results presented as mean ± standard deviation (SD). Statistical significance of the laminin-5-dependent survival results was determined by Student's t-test.
Results: The survival of both cell lines was reduced in the presence of gefitinib; moreover, the antitumor effect of gefitinib was abrogated when cells were exposed to laminin-5 (see table), an effect that was dose dependent with regard to laminin-5. To determine the cellular pathways involved in gefitinib resistance, we investigated the proliferation and survival pathways of HLF and Alexander cells. Only the level of pAkt expression was inhibited by gefitinib and partially restored by pre-exposure to laminin-5.

Cell lines	Gefitinib	% survival ±SD		Statistical
	IC_{50} (μM)	Gefitinib (IC_{50})	Gefitinib (IC_{50}) + laminin-5 (1 μg/mL)	significance
Alexander	0.67±0.22	64.9±11.1	88.3±12.9	P<0.001
HLF	4.04±0.54	62.4±8.2	84.8±7.8	P<0.001

Conclusions: We have shown that gefitinib has activity in an *in vitro* HCC model and may warrant further investigation in the clinical setting for the treatment of HCC. Determination of laminin-5 expression in tumor tissue could contribute to the cytotoxic activity of gefitinib and could result in a valid predictive factor for the choice of therapy.
Iressa is a registered trademark of the AstraZeneca group of companies

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TGFb is a major factor constitutively acting on T cells in vivo: implications for cancer immunotherapy

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Background: The significant role of TGFβ in the modulation of the immune response has been well studied in murine models and several important studies also indicate a crucial role of TGFβ in tumor immunology. TGFβ is present systemically at measurable levels in healthy individuals and is significantly increased in patients suffering from many different cancer types. To better understand the role of this important factor on T cell homeostasis and activation we explored the molecular signatures of human $CD4^+$ T cells after withdrawal or addition of this important factor.
Materials and Method: Whole genome expression profiling (HG-U133A, Affymetrix) of 64 samples from $CD4^+$ T-cells and PBMCs maintained for different time courses either in the presence or absence of TGFβ was performed. Differentially expressed genes where further evaluated using protein based assays.
Results: Primarily we withdrew the influence of TGFβ from $CD4^+$ T cells by culturing these cells under serum-free conditions. A significant number of genes changed already after 2 hrs (177 genes) and 8 hrs (203 genes). While several cellular systems showed gene expression changes, the loss of TGFβ target genes was one of the most prominent signatures observed in $CD4^+$ T cells. Of particular interest is the decrease in cell cycle regulators under TGFβ free conditions, suggesting that TGFβ plays an important role in protecting $CD4^+$ T cells from spontaneous proliferation by blocking entry into the cell cycle. Among the genes changed after TGFβ withdrawal we also identified several target genes, which were not previously described to be regulated in T cells. Addition of TGFβ to the cells at physiological conditions reversed the observed effects in single TGFβ target genes and addition of TGFβ at higher concentrations to T cells activated by polyclonal T cell stimulation lead to the identification of novel signalling cascades modulating T cell activation. The prominent effect of TGFβ on T cells – as assessed here on a comprehensive molecular level – was specific for T cells since PBMC analyzed under the same conditions responded with an overwhelming NFκ B signature which was confirmed by the identification of a NFκB p50 nuclear translocation.
Conclusion: While the inhibitory role of TGFβ on T cell activation has been previously recognized, the molecular mechanisms leading to this inhibition has not been completely resolved. Here we not only demonstrate an important role of TGFβ as a major factor keeping T cells in a "resting phenotype", but also uncover the signaling pathways involved in the inhibitory effect of TGFβ on T cell activation. The ability to specifically block inhibitory factors such as TGFβ on the molecular level will open completely new avenues to enhance active cancer immunotherapy such as cancer vaccines.

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Differential expression of biomarkers in NSCLC: a comparison between smokers and never smokers

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Background: Clinical, histopathological and molecular patterns may be different between non-small cell lung cancer (NSCLC) arising in smokers and never smokers. In the era of molecular targeted therapies, tumor response may vary depending of specific biomarker profiles. Never smokers appear to be more sensitive to EGFR inhibitors. The present study aims at comparing selected biomarkers between smoking-related and smoking-free NSCLC.
Material and Methods: 232 NSCLC formalin-fixed specimens from patients with (n=179) or without (n=53) a smoking history were collected. All patients underwent curative surgical treatment between 1995 and 2003 and were matched by sex and histologic type between smokers and never smokers. Tissue microarrays were established and immunohistochemistry was performed to analyze the expression of hTERT, Ki 67 and pAKT. For each marker, a score was established in all patients, based on the percentage of positive cells, intensity of staining and subcellular distribution. Z test, Fischer's exact test and ÷* analysis were used to compare the distributions of these scores between smokers and never smokers.
Results: NSCLC in never smokers included 50 adenocarcinomas and 3 squamous cell carcinoma (SCC). NSCLC in smokers included 158 adenocarcinomas and 21 SCC. The expression of the catalytic subunit of telomerase (hTERT) was higher in smokers as compared to never smokers ($p<0.02$). This difference remained significant when comparison between smokers and never smokers was performed according to the histologic type and a trend was observed when sex of patients was taken into account. The strongest difference was noted when the intensity of the nucleolar staining was compared between smokers and never smokers. Ki67, a marker of cell proliferation, was also more frequently expressed in tumors from smokers than in never smokers ($p<0.01$). This held true when sex and histological subtype were taken into account. In contrast, no difference was found for the expression of pAKT between smokers and never smokers. Studies analyzing the difference of expression of other biomarkers (EGFR, pEGFR, pERK) are now in progress in our department.
Conclusions: Higher levels of proliferation and telomerase activation are observed in NSCLC related to tobacco carcinogens as compared to NSCLC in never smokers. The specific differences in EGFR related pathways in this setting are currently explored and will be presented.