

phenotype showed better response rates to EGFR inhibitors than cell lines with mesenchymal characteristics. Cancer cells, that have undergone transition, are less dependent on EGFR, because of the activation of alternative growth pathways. Loss of E-Cadherin, is the first indication in the EMT. Usually, E-Cadherin is located in the cell membrane but it was described recently that cytoplasmatic location is associated with a mesenchymal phenotype for NSCLC, and even nuclear translocation occurred for other cancer types.

Materials and Methods: The expression of epithelial and mesenchymal markers, and of proteins of the EGFR downstream signal transduction pathways of 26 patient-derived xenografts was determined via Western analysis. The E-Cadherin localization within the tumor cells was investigated with immunofluorescence.

Results: One goal of this study was to find out whether EMT plays a role in the sensitivity of 26 patient derived NSCLC xenograft models to erlotinib and cetuximab. Each tumor model showed a unique expression pattern. However, a distinct classification to either an epithelial or a mesenchymal phenotype was not possible, as many xenografts expressed both, epithelial and mesenchymal markers.

In our xenografts E-Cadherin was not only localized in the membrane, but also in the cytoplasm. Therefore, cytoplasmatic translocation of E-Cadherin could not be shown clearly. Constitutive nuclear E-Cadherin localization among the 26 tumor models was not observed. A nuclear expression was only found, when the tumor cells were damaged due to apoptosis or necrosis after therapy.

Conclusion: Opposite to literature reports a correlation between the expression of epithelial or mesenchymal, and signal transduction markers to the response of the xenografts to the EGFR inhibitors erlotinib and cetuximab could not be found.

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A sensitive tamoxifen response profile in patients with metastatic breast cancer indicates that an Interferon-gamma (IFN- γ) centered cellular immune response is involved in tamoxifen resistance

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Background: Only half of the patients with advanced ER-positive breast cancer respond to tamoxifen while the other half is resistant. The aim of this study was to develop a gene signature that is predictive for response to tamoxifen treatment and to study molecular mechanisms that contribute to tamoxifen resistance.

Materials and Methods: The study was performed on 101 estrogen receptor-positive breast carcinomas from patients with metastatic disease treated with tamoxifen in first-line. Main clinical end point was the effect of tamoxifen on time until tumor progression. RNA was isolated from the primary tumor samples collected at two independent cancer clinics in the Netherlands, and hybridized to Agilent whole genome 4x44K microarrays. A training set of 68 tumors were randomly divided into training and test groups. By using a t-test and 1000 rounds of 10-fold cross validation, the best discriminating genes were identified.

Results: A 26-gene signature was developed that can identify tamoxifen non-responders with a sensitivity of 77% and specificity of 79% in the training set. The signature was validated on an independent set of 33 tumors (sensitivity 75% and specificity 71%). The difference in median TTP for the predicted groups was 9.7 months (14.6 months for responders versus 4.9 for non-responders) (log-rank $P=0.0115$). The analysis of biological function and canonical pathways indicated that genes involved in immune response were enriched in the signature. Gene network analysis revealed that an Interferon-gamma (IFN- γ) centered immune response network is upregulated in tamoxifen resistant patients.

Conclusion: A 26-gene signature was developed that can predict the response to tamoxifen treatment for patients with metastatic disease. Cellular immune response may play a critical role in tamoxifen resistance.

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MicroRNAs as circulating tumor cells biomarkers in gastrointestinal cancer

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Background: MicroRNAs (miRNA) are small noncoding RNAs with relevant posttranscriptional regulatory functions. miRNAs have potential as diagnostic biomarkers and therapeutic targets in cancer.

Objective: To identify miRNAs with diagnostic value for circulating tumor cells (CTC) detection in peripheral blood (PB) from patients with gastrointestinal (GI) cancer.

Materials and Methods: Phase I preclinical study was carried on by means of bioinformatic tools for miRNAs profiling including miRgator, miRBase, SmiRNadb, Gene-Hub Gepis, microRNA.org and miRNAMap, highly expressed in GI cancer, but absent in hematopoietic-derived sources. qRT-PCR mature miRNA Detection Kit (Ambion) was used to profile the expression of selected miRNAs. U6 and 5S were used as internal controls.

Results: In silico analysis showed a set of miRNAs highly expressed in GI cancer in relation to hematopoietic samples, including miR-141 and miR-200 family, miR-31, miR-32, miR-192 and miR-375. In order to validate the usefulness of miRNAs as molecular markers for circulating tumor cells detection, qPCR experiments were performed in GI cell lines and a cohort healthy donors and GI patients. Preliminary analysis of GI cancer PB showed higher relative expression levels for selected miRNAs comparing with age-matched controls' PB.

Conclusion: Our results indicate that miRNA bioinformatic approach is an useful method to select GI cancer-associated miRNAs. This miRNA profiling in PB should be further validated as markers for CTC detection. Supported by grants PI06/1541 and PI07/0477 from Fondo de Investigaciones Sanitarias (FIS), Instituto de Salud Carlos III. S. Díaz Prado is beneficiary of an Isidro Parga Pondal contract from Xunta de Galicia (Spain).

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KRAS mutation is highly correlated with EGFR alterations in patients with non-small cell lung cancer

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Background: The activation of epidermal growth factor receptor (EGFR) pathways that mediate a variety of cellular responses, including cell division, invasion, and cellular repair, are very important to the carcinogenesis of non-small cell lung cancer (NSCLC). A major downstream signaling route of the EGFR is via the RAS-RAF-MAP (mitogen-activated protein) kinase pathway. Activation of RAS initiates a multistep phosphorylation cascade that leads to the activation of MAPKs in cancer cells. Up to the present date, it remains controversial about the co-existing mutations of KRAS and EGFR, and the co-existence of KRAS mutations and EGFR overexpression in NSCLC.

Materials and Methods: In order to elucidate KRAS and EGFR gene alterations, and their association among NSCLC patients in Taiwan, we analyzed for mutations of KRAS and EGFR genes in surgically NSCLC to determine the prevalence of these mutations in Taiwanese lung cancer patients. In addition, we examined the relationship between the mutations and clinicopathologic features of NSCLC patients by direct sequencing and Northern blotting.

Results: Of 72 cancerous tissues, EGFR mutation was present in 17 samples (24%) and EGFR overexpression was detected in 26 samples (36%). The KRAS mutation was found in 39% (28/72) of the tissue samples. The co-existing mutations of KRAS and EGFR were found in only 1.4% (1/72) of the samples; the co-existence of EGFR mutation and overexpression was found in 16 samples (22%).

Conclusion: These results described above showed that the mutations of KRAS and EGFR would not co-exist regardless of which clinical stage or tumor size the patients experienced or whether the presence of metastasis. Also, nearly no co-existence was found between KRAS mutation and EGFR overexpression. These outcomes greatly assist the prediction on the efficacy of the current anti-EGFR therapeutic targeted drugs for cancer patients.

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Irradiation-enhanced mammalian target of rapamycin (mTOR)-targeted glioblastoma therapy with CCI-779 (temsirolimus)

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Background: The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway plays a critical role in oncogenesis, and dysregulation of this pathway is particularly common in human malignant gliomas. In these tumors, activation of PI3K/AKT/mTOR signaling leads to cell cycle progression, neovascularization, escape from apoptosis and inhibition of autophagy, and is associated with poor prognosis. CCI-779 (temsirolimus), a soluble ester analogue of rapamycin, is a small-molecule inhibitor of the mTOR kinase that has been demonstrated to have some antitumor activity. Given that postoperative radiochemotherapy is the standard of care in the first-line

treatment of glioblastoma, this translational project aims to analyze whether CCI-779 improves the efficacy of irradiation in experimental glioma models in vitro and in vivo and whether the phosphatase and tensin homolog (PTEN) is of predictive value.

Materials and Methods: Six human glioblastoma cell lines and two glioblastoma-initiating primary cultures were tested in clonogenicity, matrigel invasion and cell viability assays, and flow cytometry-assisted analyses of cell cycle, autophagy and apoptosis. Human cerebral microvascular endothelial cells (HCMEC) and measurements of the vascular endothelial growth factor (VEGF) were used to determine antiangiogenic activity. Immunosuppressive effects were evaluated in mixed leukocyte reactions (MLR). The PTEN status was analyzed by cDNA sequence analysis, methylation-specific polymerase chain reaction of the *PTEN* promoter region, immunoblots of PTEN and its downstream target phospho-AKT, and PTEN immunohistochemistry.

Results: We observed that CCI-779 applied at effective but not yet immunosuppressive concentrations exerted marked antiproliferative, proautophagic, anticlonogenic and antiangiogenic activity that was enhanced when combined with irradiation. Moreover, CCI-779, when applied following radiosensibilization, inhibited invasiveness in a supra-additive way and reversed the known proinvasive effect of sole sublethal irradiation. The responsiveness towards CCI-779 could not be correlated with the PTEN status. In currently ongoing MRI-monitored, syngeneic, orthotopic glioma experiments, we assess whether a treatment approach of combined CCI-779 and irradiation would also result in antitumoral activity in vivo.

Conclusion: In summary, these results support the clinical evaluation of combined targeted mTOR inhibition with CCI-779 (Torisel™) and radiotherapy in patients with newly diagnosed glioblastomas.