differentially regulated between Ha-RAS and Ki-RAS leading to Ha-RAS specific induction of migrative phenotype. We demonstrate that the AP-1 sites in vimentin promoter are involved in the regulation of vimentin and FRA-1 binds to vimentin promoter in vivo, regulates its expression as well as migration and invasion properties (3).We identified TAF12 levels as being up-regulated in cell lines bearing natural RAS mutations or stably overexpressing a mutated RAS isoform and was dependent on the MEK pathway. We further identified a functional ETS binding site on the TAF12 promoter. Reduction of TAF12 levels by siRNA treatment enhanced E-cadherin mRNA and protein levels and reduced migration and adhesion properties of RAS transformed cells with Epithelial to Mesenchymal Transition (4).

Conclusions. Overall, our study has identified a signature of metastatic gene expression in colon and reveals new mechanisms of regulation of the two major EMT related genes, that of vimentin and e-cadherin by Fra-1 and TAF12 transcription factors respectively

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Role of COX-2 and Ras activation in pancreatic adenocarcinogenesis

S. Chiblak[1], I. Esposito[2], A. Nieters[3], B. Hoeft[3], K. Mueller-Decker[1]
[1]Deutsches Krebsforschungszentrum, Core Facility Tumour Models, Heidelberg, Germany; [2] Technical University of Munich, Institute of Pathology, Munich, Germany; [3] Deutsches Krebsforschungszentrum, Molecular Tumour Epidemiology, Heidelberg, Germany

Since the majority of pancreatic adenocarcinomas are highly aggressive and therapeutically non–accessible, basic research on pancreatic carcinogenesis is crucial. Cyclooxygenase-2 (COX-2), a key enzyme of prostaglandin (PG) biosynthesis, is over-expressed in 75 % of carcinomas including those of the pancreas. In our group, the pathologic and molecular changes of transgenic mouse pancreas with keratin 5 promoter-driven over-expression and activity of COX-2 were characterized. Transgenic pancreata developed cystic intra-ductal papillary mucinous neoplasms resembling human lesions in this organ. Multiple inflammatory clusters comprised of B- and T-cells as well as macrophages, were found to be spread throughout the pancreata. Mutational activation of the K-Ras gene, predominantly in codon 12, is known to be an initiating event in human pancreatic adenocarcinogenesis. Pyrosequencing of DNA from transgenic pancreatic cysts for mutations in cancer-relevant codons 12, 13, and 61 of this gene revealed wild-type sequences. Nevertheless, activation of Ras (measured as increased levels of GTP-Ras) and Ras-downstream effector kinases such as Mitogen-Activated Protein Kinase (MAPK) and AKT was enhanced. Celebrex treatment of transgenics suppressed the accumulation of PG, the activation of Ras, MAPK, AKT, the pathologic changes, including the inflammatory phenotype. Analysis of PGE2 receptors EP1-4 in pancreata of transgenic mice showed an over-expression of EP-1 and EP-4 as compared to wild type organs, while EP-2 and EP-3 expression was not modulated. By indirect multi-colour immunofluoresence stainings all receptors were located in the keratin 19-positive pancreatic ducts, in macrophages, and with the exception of EP-2, in CD31-decorated blood vessels. EP-1 was only observed in CD45/B220- and CD4-positive lymphocytes. In ongoing studies the role of individual EP receptors in pancreatic carcinoma cells with respect to proliferation, migration, and Ras signaling is studied. In conclusion, there is strong evidence for a causal relationship between aberrant COX-2 expression, COX-2-mediated PG signaling via Ras, and the development of the pre-invasive lesions including the inflammatory phenotype.

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Antitumoural effect of cannabinoids in an animal model of breast cancer

M. Muñoz Caffarel[1], C. Andradas[1], E. Mira[2], C. Cerutti[1], S. Mañes[2], M. Guzmán[1], C. Sánchez[1]
[1]Universidad Complutense de Madrid, Dept. Biochemistry and Molecular Biology I, Madrid, Spain; [2] Centro Nacional de Biotecnología/CSIC, Dept. Immunology and Oncology, Madrid, Spain

We have previously shown that Δ^9-tetrahydrocannabinol (THC), the most important cannabinoid in terms of potency and abundance, reduces human breast cancer cell proliferation in vitro by blocking the progression through the cell cycle and by inducing apoptosis. Here we show that cannabinoids have antitumoural properties in an animal model of breast cancer: the MMTV-neu mouse. These transgenic mice carry an inactivated neu oncogene under the transcriptional control of the mouse mammary tumour virus promoter/enhancer and, as a consequence, they develop mammary tumours with a latency period of approximately 7 months. Mice were palpated twice weekly for the detection of mammary gland nodules. At the time of appearance of the first tumour, cannabinoid peritumoural treatment was started and maintained for three months (twice per week). Tumour volume was measured during this period. Our results show that both THC and JWH-133, a selective ligand for the CB_2 -non psychotropic-cannabinoid receptor, drastically reduce tumour growth and the number of tumours per animal. The presence of cannabinoid receptors in these tumours was confirmed by confocal microscopy and real-time quantitative PCR. In order to elucidate the mechanism of cannabinoid antitumoural action in this model, we performed different experiments in (i) tumour samples, (ii) cells isolated from tumours, and (iii) an established human breast cancer cell line that naturally overexpresses neu (SkBr3). Preliminary data indicate that the mechanism underlying cannabinoid effect include inhibition of proliferation, metastasis and angiogenesis, together with a modulation of tumour immune infiltration.

In summary, our results show for the first time that cannabinoids have an antitumoural effect in a genetic model of cancer, and confirm the potential of these compounds as anticancer therapeutic tools.

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Mechanisms of apoptosis and cell cycle arrest in sub-cutaneous breast tumours treated sequentially with doxorubicin followed by zoledronic acid

P. Ottewell[2], R.E. Coleman[1], I. Holen[2]
[1]University of Sheffield, Clinical Oncology, Sheffield, United Kingdom; [2] University of Sheffield, Academic Department of Clinical Oncology, Sheffield, United Kingdom

Background: Late stage breast cancer involving metastasis to the bone is often treated with a chemotherapeutic agent in combination with the anti-resorptive drug zoledronic acid (Zol). We have previously reported that administration of doxorubicin (dox) 24h prior to zoledronic acid inhibits intra-osseous breast tumour growth, inhibits tumour cell proliferation and increases apoptosis in vivo. This is the first report of the potential molecular mechanisms by which doxorubicin and zoledronic acid exert their synergistic anti-tumour effects.

Materials and methods: MDA-MB-436-GFP cells were inoculated into the right flank of female MF1 nude mice (n=3/array). Mice were treated 1x per week for 6 weeks with saline, 2mg/kg dox, 100μg/kg zol, dox and zol simultaneously, dox followed 24h later by zol, or zol followed 24h later by dox. Animals were sacrificed 24h following final treatment. Biotin labelled RNA from each group was hybridised to a GEArray cell cycle pathway specific microarray. Genes that showed a 2 fold or greater change in expression were considered relevant, and changes were confirmed by qPCR, and Western blot.

Results: Molecular analysis of subcutaneous MDA-G8 tumours showed no effect on tumour cell cycle or apoptosis following administration of 100ug/kg zol. 2mg/kg dox caused a cell cycle block at G1-S with a down regulation of cyclin E/CDK2; whereas apoptosis-related genes were unaffected. However, when dox was administered 24h prior to zol cell cycle progression was further suppressed, was accompanied by a down regulation of cyclins E1, B, D1 and D3 as well as their related cyclin dependent kinases CDK2, CDC2, CDK4 and CDK7 compared with dox alone. Tumours treated sequentially with dox then zol also showed an induction in the apoptotic pathway, with an up regulation in Bax, a down regulation in Bcl2 and an increase in caspase 3 cleavage.

Conclusions: This is the first report showing that sequential treatment of sub-cutaneous breast tumours in vivo with doxorubicin followed by zoledronic acid induces changes in a number of specific genes associated with regulation of the cell cycle and apoptosis.

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ERK1/2 as a modulator of the cross-talk between VEGFR-2 and S1P-receptor signalling pathways in follicular thyroid ML-1 cells

N. Bergelin[1], S. Balthasar[2], K. Törnquist[3]
[1]Abo Akademi University, Turku Graduate School of Biomedical Sciences, Turku, Finland; [2] Abo Akademi University, Department of Biology, Turku, Finland; [3] Minerva Research Institute, Unit of cellular physiology, Helsinki, Finland

The sphingosine 1-phosphate receptors (S1P1-3, 5) and the vascular endothelial growth factor receptor 2 (VEGFR-2) interact in the follicular thyroid carcinoma cell line ML-1. In addition to secreting substantial amounts of VEGF-A and –C, ML-1 cells also express receptors for VEGF (VEGFR-2), opening the possibility for autocrine signalling with VEGF. We have previously shown a complex interplay, in part dependent on Akt,

between VEGF and S1P signalling in the migration and proliferation of ML-1 cells.

We now show that S1P signalling regulates VEGFR-2 protein expression. In ML-1 cells transduced with a dominant negative sphingosine kinase (SK^{G82D}), the VEGFR-2 protein expression is slightly lower than in Mock transduced cells. Over-expression of SK (SK^{WT}) induces a higher protein expression than found in Mock transduced cells. However, this up-regulation in receptor expression is not mediated by transcriptional regulation.

Preliminary data suggests that in ML-1 cells S1P-receptors (S1P1-3, 5) form complexes with VEGFR-2, both in the presence and absence of serum. Interestingly, VEGFR-2 protein expression is regulated by ERK1/2 in SK^{WT} expressing cells as well as in native ML-1 cells. Inhibiting VEGFR-2 in native ML-1 cells also inhibited S1P-induced ERK1/2 phosphorylation. Similar results were found in SK^{WT}-expressing cells.

We have previously shown that through the activation of novel and classical isoforms of PKCs (i.e. PKC-α) and subsequent activation of SK, ERK1/2 may be phosphorylated, resulting in the induction of migration. Interestingly, ML-1 cells display at least two migratory pathways, differing in their sensitivity to VEGFR-2 inhibition. We have previously shown that S1P-induced migration is sensitive to VEGFR-2 inhibition; however, an OAG induced migratory response is insensitive to inhibition of VEGFR-2, indicating that a receptor complex may indeed mediate the S1P-induced migration.

ML-1 cells secrete substantial amounts of VEGF-A, which can be stimulated by micromolar concentrations of S1P. Although, transducing ML-1 cells with SK^{G82D} or SK^{WT} did not affect basal VEGF-A secretion, inhibiting ERK1/2 significantly reduced the VEGF-A secretion of SK^{WT}-cells as well as of Mock-transduced cells. We conclude that ERK1/2 plays a major role in the cross-talk between the signalling pathways of S1P- and VEGF-receptors. Taken together ERK1/2 regulates both the expression of VEGFR-2 and VEGF-A secretion in SK^{WT} cells.

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Significant down-regulation of DNA repair systems in non-small cell lung tumours that reactivate telomerase

C. Frías[1], P. Ortega[1], A. Morán[1], C. De Juan[1], T. Fernández-Marcelo[1], A. Gómez[2], F. Hernando[2], A.J. Torres[2], M. Benito[1], P. Iniesta[1]
[1]Facultad de Farmacia, Universidad Complutense, Bioquímica y Biología Molecular II, Madrid, Spain; [2] Hospital Clínico San Carlos, Servicio de Cirugía II, Madrid, Spain

Telomere function and DNA damage response pathways are frequently inactivated in cancer. Moreover, some telomere-binding proteins have been implicated in DNA repair. The main aim of this work consists of evaluating possible relationships between telomere dysfunction and DNA repair systems in non-small cell lung cancer (NSCLC).

We analysed 83 NSCLCs and their corresponding control samples obtained from patients submitted to surgery. Telomere function was evaluated by determining telomerase activity and telomere length. DNA repair expression assays were established by using cDNA arrays containing 96 DNA-repair genes and by Real Time Quantitative PCR.

Our data indicated that 83.13% of tumours showed telomerase activity. We observed significant associations between enzyme activity and TNM stage (P = 0.008), size (P = 0.041) and histology of tumours (P = 0.001). Also our results revealed that shorter telomeres were significantly associated with tumours that had grown into the area of mediastinum or cancers with a malignant pleural effusion (P = 0.003). In relation to expression assays, we detected a group of DNA repair genes whose expression levels were significantly associated with telomerase activity. As expected, TERT expression (P = 0.044) was significantly increased in the group of tumours displaying telomerase activity. However, expression data for DCLRE1C (P = 0.001), GTF2H1 (P = 0.009), PARP3 (P = 0.005) and MLH1 (P = 0.003) indicated a significant down regulation in association with telomerase activity. Moreover, TRF2 was down regulated in telomerase positive tumours showing significant telomere shortening (P = 0.042).

In conclusion, results here presented suggest an association between the loss of several DNA repair genes and telomerase activity, which may be of relevance in the pathogenesis of non-small cell lung cancer.

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MKP1 regulates susceptibility to genotoxic stress

V. Rodriguez Fanjul[1], I. Sanchez-Perez[1], R. Perona Abellon[1]
[1]Biomedical Research Institute, Departamento de Modelos Experimentales de Enfermedades Humanas, Madrid, Spain

Dual-specificity phosphatase type 1, DUSP1/MKP1, is a member of the dual-specific family of phosphatases that dephosphorylates MAPKs, including ERK, JNK and P38. MKP1 is a nuclear protein, whose basal levels are low in unstressed or unstimulated cells and its expression is induced following stimulation with mitogens, oxidative stress, hypoxia, and DNA damaging agents. Additionally, different studies have shown that MKP1 is overexpressed in different types of cancer (breast, ovarian, prostate and lung carcinoma). Many chemotherapeutic drugs induce apoptosis in cancer cells as a consequence of activation of JNK and p38 pathway. The ability of MKP1 to decrease the activity of these kinases results in protection from apoptosis and drug resistance. Resistance to radiation and chemotherapy is one or the major obstacles in cancer treatment. Thus, interference of MKP1 may be an alternative strategy for manipulating MAPK pathways in a cell-type specific manner. Indeed, previous work in our group has shown that inhibition of MKP1 expression sensitizes non-small-cell lung cancer (NSCLC) to cisplatin.

In this work, we have investigated the role of MKP1 in modulating antitumoral-induced apoptosis. Mouse embryonic fibroblasts (MEFs) derived from wild-type (MEF+/+) and MKP1 knock-out (MEF-/-) mice were exposed to different drugs commonly used in the clinic. Cell viability was studied by crystal violet staining method; MAPK activity, c-jun, caspase-3 and MKP1 expression levels were determined by inmunoblotting, using specific antibodies. MEF+/+ cells treated with alkylating agents showed a direct correlation between MKP1 expression and JNK or p38 inactivation, and in turn a lower sensitivity to drugs compared to MEF-/-. In addition JNK and p38 activity was strongly activated in MEF-/- and the cells were hypersensitive to these drugs. On the other hand, no differences were observed in either sensitivity or MAPK activity between MEF+/+ or MEF-/- after treatment with agents that induce double strand breaks; either agents targeting cytoskeleton; or drugs blocking DNA synthesis, which are not able to induce MKP1 expression.

Our results strongly suggest that MKP1 specifically regulates survival in response to alkylating agents by modulating JNK and p38 activity implicating MKP1 as an important mediator of chemoresistance. Therefore, pharmacological inhibition of MKP1 could be used in combination with alkylating drugs to induce chemosensitization and overcome chemoresistance.

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SK3 channel promotes melanoma cell migration

A. Chantome[1], A. Girault[1], M. Potier[1], P. Vaudin[2], C. Collin[2], M.L. Jourdan[1], J.C. Pagès[2], P. Bougnoux[1], C. Vandier[1], V. Joulin[3]
[1]INSERM U921, Université François Rabelais, Nutrition Growth and Cancer, Tours, France; [2] ERI19 INSERM EA3856, Université François Rabelais, Virus Pseudovirus: Morphogenesis and Antigenicity, Tours, France; [3] Institut Gustave Roussy, Genome and Cancer, Villejuif, France

Numerous studies have demonstrated that potassium channels interfere with pathways controlling the balance between cell growth and cell death. In contrast, the role of potassium channels in tumour cell dissemination and metastasis has been less intensively investigated. Among potassium channels we recently found that SK3 channel, a member of apamin-sensitive small-conductance calcium activated potassium channels (SK_{Ca}), is a mediator of breast cancer cell migration[1]. Since melanoma is an extremely aggressive disease with metastatic potential, we investigated if SK3 channel is expressed in melanoma cell lines and if this channel plays a role in melanoma cell migration.

To investigate the presence of SK3 channel in melanoma cells we first performed RT-PCR and Western blot analyses in three human melanoma cell lines, SKmel28, Bris and 518A2. We found that SK3 gene expression and proteins were detected in Bris and 518A2 but not in SKmel28 cells. Then, using apamin a specific blocker of SKCa channels, we compared the migration behaviour of melanoma cell expressing or not SK3 protein. This blocker reduced migration of Bris and 518A2 cells but didn't affected migration of SKmel28 cells. Consequently, apamin reduced migration only in cells expressing SK3 protein. To fully demonstrate the contribution of SK3 channel in melanoma cell migration we have enforced SK3 gene expression in SKmel28 cells and knocked-down SK3 transcripts in Bris and 518A2 cells using lentiviral vector containing respectively a SK3 cDNA and a shRNA-SK3. Western blot experiments confirmed a large decrease of SK3 protein in Bris and 518A2 cells and a SK3 protein expression in SKmel28 cells. Patch-clamp recordings demonstrated that if silencing SK3 expression depolarised plasma membrane of Bris and 518A2 cells, stable expression of SK3 protein hyperpolarised membrane potential of SKmel28 cells. In parallel, we found that expression of SK3 gene in SKmel28 cells increased their migration and depletion of SK3 gene in 518A2 and Bris cells decreased their migration. In contrast to numerous potassium channels, in our case, SK3 channel seems to not interfere with cell proliferation or cell death.

In conclusion and as observed for breast cancer cells, SK3 channel is a mediator of melanoma cell migration. Moreover, these new results suggested that SK3 channels promote cancer cell migration by hyperpolarising plasma membrane leading probably to subsequent Ca^{2+} influx. To go further we will study SK3 involvement in tumour growth and/or