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

1. Roberts, M., Drosopoulos, K., Vasileiou, G., Stricker, M., Taoufik E., Maercker, C., Guialis, A., Alexis, MN. and Pintzas, A. (2006). Int. J. Cancer 118, 616–627.

2. Joyce et al. In preparation.

3. Andreolas, C., Kalogeropoulou, M., Voulgari, A. and Pintzas, A.(2008). Int J Cancer. 122, 1745–1756.

3. Voulgari, A., Voskou, S., Tora, L., Davidson, I. Sasazuki, T., Shirasawa, S., and Pintzas, A. (2008). Under Revision.

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Role of COX-2 and Ras activation in pancreatic adenocarcinogenesis

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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

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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

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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

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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,