dependent mast cell transformation. In theses cells, we have identified interaction partners of phosphotyrosine 568 and 570 using affinity pull down assays with synthetic phosphopeptides followed by mass spectrometry identification. Identification of ten partners (SHP-1, SHP-2, SHIP, Stat5, Grb2, CrkL, PLCγ1, PLCγ2, Fes and Syk) was confirmed by western blotting and the in cellulo relevance of these interactions was emphasised by co-imunoprecipitation with Kit in HMC-1 cells.

In an attempt to confirm the binding specificity of these partners to the dityrosine motif, we have mutated both tyrosines to phenylalanine and introduced the mutant receptor in Ba/F3 cells. Some partners described still coimmunoprecipitate with Kit in the cells stably transfected with the mutant receptor Kit Y568F-Y570F-D816V, suggesting a redundancy of binding sites. However, this dityrosine motif has shown to be the only docking site for the tyrosine kinase Syk, as coimmunoprecipitation is lost with mutant receptor.

We are now addressing the function of this complex using RNA interference. We have successfully silenced Syk expression in HMC-1 cells and we are now focussing on the functional and biochemical consequences of Syk depletion. In the context of Kit oncogenic activation, we aim to define whether the kinase Syk is implicated in proliferation, in adhesion to fibronectine, in migration towards SCF in a Boyden chamber or in degranulation.

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Cell-cell communications in a breast cancer senescence model

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BACKGROUND: The tyrosine kinase receptor ERBB2 is a key receptor in the development of breast cancer and demonstrates an aggressive ability to transform cells via mitogenic and anti-apoptotic signals. However, recent evidence suggests premature senescence of cells undergoing mammary tumourgenesis through the ERBB2 pathway is a type of anti-carcinogenic programme. The over expression of ERBB2 induces the up-regulation of cell cycle inhibitors triggering senescence in breast cancer cells. Intracellular and intercellular communication plays a prominent role in the cell cycle and in particular cell cycles in cancer. Direct cell-cell contact through the formation of gap junctions is of major interest in investigating senescence of tumour cells. Over the past decade, microdevices have become increasingly popular tools for addressing key questions in the life sciences. We have developed a cell-cell contact microdevice to enable contact modes of communication between breast cancer cells to be characterised.

MATERIALS AND METHODS: Doxycycline induces senescence in the breast cancer cell line MCF7 carrying the ERBB2 variant vector NeuT (MCF7/NeuT). Cells were cultured with and without doxycycline on custom made individual cell culture plates. Once doxycycline had induced senescence these cells were interfaced with untreated and non-senescent cells using the contact microdevice. The distance between the cell culture plates, in both lateral and vertical dimensions, was less than 10μm to allow for sufficient cell-cell interaction.

RESULTS: The uninduced MCF7/NeuT cells proliferated and were in contact with the senescent MCF7/NeuT cells. Senescence was observed in induced cells when the NeuT gene was switched on by doxycycline. This was confirmed by multiple cell protrusions, granular structures and flattened cell morphology as well as cell cycle arrest. Also, the cyclin-dependent kinase inhibitor p21 was shown to be up-regulated and centralised to the nucleus. The knock-down of p21 abolished cell cycle arrest allowing for tumourgenesis to progress.

CONCLUSIONS: In these first contact experiments we have demonstrated the potential for the cell-cell contact microdevice to be used to bring two different cell types into contact for short periods (1 min to 4 hours). Presently, we are studying the effect of such a transient contact between senescent and native MCF7 cells to determine the possibility of a 'kiss of senescence'.

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Cell type-specific methylation of the SNCG and S100A4 genes and their relation to expression changes in urothelial cancer

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Hypomethylation of repeat DNA sequences is common in human cancers, but only a few single-copy genes have been reported to be activated by DNA hypomethylation, including SNCG and S100A4. Since both genes were suggested to be overexpressed in urothelial cancers by microarray expression studies performed in our institution, we wondered whether the increases were caused by hypomethylation. We compared expression in 13 urothelial carcinoma (UC) cell lines and cultured normal urothelial cells as well as in tumor and benign tissues, analyzed the effect of the methylation inhibitor 5-aza-2-deoxycytidine (azadC), and determined methylation patterns by bisulfite sequencing, also in fibroblasts and blood cells. Of 13 UC lines, 6 showed SNCG overexpression vs. normal cells, but 7 very low levels. Similarly, in carcinoma tissues, both increased and strongly diminished SNCG expression were evident. Treatment with azadC restored expression in UC lines with undectable mRNA levels. In these lines, the SNCG promoter was densely methylated, whereas it was unmethylated in normal urothelial cells and in UC lines with elevated gene expression. Fibroblasts, blood leukocytes and ureteral connective tissue showed partial methylation. Similarly, the SNCG was unmethylated and expressed in normal prostate epithelial cells, but densely methylated and silenced in prostate cancer cell lines. S100A4 was expressed more strongly in six UC lines than in normal cells, but was undetectable in three cell lines, in which azadC treatment increased expression. Fibroblasts displayed high S100A4 expression. Methylation of the promoter and an intronic regulatory site was accordingly lowest in connective tissue and blood cells, whereas all cells of urothelial origin showed at least partial and often heterogeneous methylation with moderate correlation to expression. No significant overall difference in S100A4 expression was found between benign and cancerous bladder tissues. Our data identify SNCG and S100A4 as new cell type-specific methylated genes. SNCG downregulation in some urothelial carcinomas is associated with hypermethylation, whereas upregulation in other cases occurs independent of methylation changes at the promoter. S100A4 methylation is likewise cell type-specific. Interestingly the gene can be expressed despite substantial methylation at its promoter, likely due to a low density of methylatable CpG-sites. Of note, our findings suggest caution in ascribing overexpression of the two genes in cancers to DNA hypomethylation, without considering changes in the cellular composition of the tumors. For instance, apparent hypomethylation and overexpression of SNCG might reflect an increased proportion of carcinoma cells, whereas apparent hypomethylation and overexpression of S100A4 might result from an increased proportion of fibroblasts.

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EGR1 expression in breast cancer cells exposed to PPARg agonists occurs in a PPARg-independent pathway

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Both natural (Prostaglandin PGJ2) and synthetic ligands (Troglitazone (TGZ), Ciglitazone (CGZ), Rosiglitazone (RZG)) of PPARγ nuclear receptors inhibit the growth of several cancerous cell lines. Several studies suggest that these agonists possess PPARγ-independent effects. Indeed, Baek et al have shown that TGZ induced the expression of the early transcription factor EGR1 (Early Growth Response gene 1) followed by the expression of the pro-apoptotic Growth Differentiation Factor 15 (GDF15) in human colon cancer cells. In this report, EGR1 induction appeared to be independent of PPARγ because this event was not blocked by the PPARγ antagonist GW 9662 and not induced by other PPARγ ligands (Baek et al, 2004). Based on this study, we aim at characterizing the potential PPARγ - independent signaling pathway of PPARγ agonists in the breast cancer cell line MCF7. We are focusing on the early response of the cells involving phosphorylation pathways.

Cells were treated with PGJ2 and the synthetic ligands TGZ, CGZ, RGZ. Kinetics and dose-dependent induction of EGR1 mRNA expression were studied. EGR1 mRNA level peaked after 3 hours of incubation with 25 microM TGZ, CGZ and PGJ2 and then gradually decreased. RGZ did not show this effect. The increase in EGR1 protein level in MCF7 cells was observed by immunofluorescence. In contrast to the study in colon cancer cells, most of the PPARγ ligands induced EGR1 expression in MCF7 but did not lead to the increase in GDF15 mRNA level.

EGR1 induction by PPARγ ligands still occurred in cells co-treated with PPARγ antagonists (GW9662, T007) suggesting that PPARγ receptors were not involved in the early response. This was further confirmed by the induction of EGR1 mRNA using the non-PPARγ ligand, derived from TGZ, delta2-TGZ. Moreover, MEK/ERK inhibitors (PD098059, U0126) abolished the EGR1 mRNA induction by TGZ, CGZ and PGJ2. Furthermore, 5 min TGZ-treatment of the cells induced the phosphorylation of ERK1/2.

Overall, these results demonstrate that natural and synthetic PPARγ agonists, with the exception of RGZ, induce the activation of MAP Kinases followed by the expression of the early transcription factor EGR1. This early response is independent of PPARγ receptors and the mechanism of activation of the MAP Kinase pathway need to be elucidated. It would be interesting to determine if EGR1 induction is involved in a later PPARγ-independent event: the proteasomal degradation of the estrogen receptor alpha.