Distinct p53 response profiles in transgenic mouse models of thyroid-specific PBF and PTTG expression

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3.72±0.32, respectively, $P<0.001$), had decreased proliferation in pancreatic (0.35±0.03% (pasireotide) vs. 0.78±0.08% (PBS)) and pituitary (0.73±0.07% (pasireotide) vs. 1.81±0.15% (PBS)) NETs (both $P<0.0001$), but had increased apoptosis in pancreatic (0.42±0.05% (pasireotide) vs. 0.19±0.03% (PBS)) and pituitary (14.75±1.58% (pasireotide) vs. 2.35±0.44% (PBS)) NETs (both $P<0.001$). Thus in summary, pasireotide treatment increased survival by ~20% and inhibited pancreatic and pituitary NET growth, indicating its potential as an anti-proliferative and pro-apoptotic therapy for pancreatic and pituitary NETs.

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P147

Steroid sulphatase and G-protein coupled oestrogen receptor in human colorectal cancer: correlation with late-stage disease and potential therapeutic targets

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Steroid sulphatase (STS) liberates sulphated oestrogens into their active forms. In the colon, evidence suggests that although initially pro-apoptotic in healthy mucosa, once malignancy occurs, oestrogens may stimulate colorectal cancer (CRC) proliferation. Moreover, greater intratumoural oestrogen synthesis is negatively associated with survival outcomes in CRC patients. However, little is known about oestrogen metabolism pathways in CRC, and whether alterations in local oestrogen synthesis and actions relate to clinical and pathological features. Furthermore, it is unknown whether manipulation of oestrogen pathways has therapeutic potential. Therefore, using qRT-PCR and immunoblotting, in healthy human colorectal tissue matched with CRC samples (n=56) we correlate the dysregulation of key oestrogen synthesis enzymes (steroid sulphatase (STS), 17β-hydroxysteroid dehydrogenase (17βHSD) type-1, type-2, type-7, and type-12) and the G-protein coupled oestrogen receptor (GPER), with patient TNM staging, lymph node infiltration, and distant metastases. In addition, ELISA assays were undertaken to ascertain the effects of oestrogens on proliferation of CRC cell lines. STS activity, 17βHSD7, and 17βHSD12 expression all showed a positive correlation with TNM staging in patient CRC samples, indicating greater oestrogen availability is linked to advanced stage disease. Increased GPER expression also significantly ($P<0.05$) correlated with late-stage malignancy. In CRC cell lines, over-expression of STS significantly ($P<0.01$) increased cell proliferation when treated with sulphated oestrogens. This effect was completely ablated when treated in combination with the STS inhibitor STX64 ($P<0.001$). Furthermore, we show here for the first time that the GPER agonist, G1, also stimulated CRC proliferation; with both oestrogen and G1 effects significantly inhibited with the GPER selective antagonist G15 ($P<0.001$). Increased STS activity and GPER expression are associated with late-stage CRC, strongly suggesting a role for oestrogens in this malignancy. Thus, reducing the availability and action of oestrogens by inhibiting STS and GPER, respectively, may have therapeutic benefits for patients with CRC.

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P148

PTTG is phosphorylated at residue T60 and regulates p53 stability, in conjunction with PBF, in head and neck cancer

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PTTG is a multifunctional proto-oncogene, overexpressed in thyroid, pituitary and other endocrine cancers. PTTG is also implicated in the pathogenesis of head and neck cancer, where high PTTG expression independently correlates with advanced tumour stage and reduced disease-free survival. Recently, abrogation of residue threonine-60 (T60) has been associated with altered PTTG half-life, chromosomal instability and cell invasion. We therefore generated a phospho-specific antibody against T60 PTTG. Transient transfection of wild-type PTTG resulted in a significant increase in T60-phosphorylated PTTG protein expression in HeLa cells (3-fold, p=0.001), which was blocked by a phospho-peptide. Detection was also lost with a PTTG-T60 mutant (T60A). Antibody specificity was further confirmed by immunoprecipitation assays. Paraffin-embedded formalin-fixed tumour sections were obtained for immunohistochemical analysis from patients with primary oropharyngeal squamous cell carcinoma. Abundant total PTTG protein expression was evident both in the cytoplasm and nucleus. In contrast, expression of T60-phosphorylated PTTG was predominantly nuclear. As interaction with its binding partner PBF facilitates PTTG nuclear localisation, and both proto-oncogenes alter p53 stability and function, we assessed the relative contributions of PTTG and PBF to p53 stability. Preliminary experiments demonstrated that transfection of wild-type PBF or PTTG into HPV-positive 93-VU-147T HNSCC cells decreased p53 protein levels compared to controls. Further, half-life studies demonstrated reduced p53 stability in 93-VU-147T cells transfected with either PBF or PTTG. Interestingly, transfection with a PBF mutant incapable of PTTG interaction, or a PTTG mutant unable to bind PBF, resulted in an initial decrease in p53 stability followed by subsequent stabilisation. These data indicate a potential role for both PTTG and PBF in modulation of p53 stability in head and neck cancers. Furthermore, PTTG is phosphorylated at residue T60 in head and neck tumours, which may alter its well described mitotic regulatory function.

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P149

Distinct p53 response profiles in transgenic mouse models of thyroid-specific PBF and PTTG expression

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Functional disruption of the tumour suppressor p53 has a critical role in promoting the development of most cancers. The proto-oncogenes PBF and PTTG1 both regulate p53 activity, but the relative contribution of each gene in influencing p53 function has not been delineated, especially in thyroid cancer where both proto-oncogenes are commonly overexpressed. To better understand the interplay between PTTG1, PBF and p53 in vivo, we examined p53 responses in primary thyrocytes cultured from transgenic mice overexpressing PBF (PBF-Tg) and PTTG1 (PTTG-Tg), either singly or in combination in a bi-transgenic murine model (Bi-Tg). Western blotting showed that p53 and γ-H2AX protein levels were elevated in PTTG1-Tg and BI-Tg thyrocytes (>2-fold; $P<0.05$). In contrast, no significant increase was observed in p53 or γ-H2AX levels in PBF-Tg thyrocytes compared to WT (P=NS). Consistent with this, a greater proportion of a panel of p53-responsive DNA repair genes were significantly down-regulated in PTTG1-Tg (30/83 genes) and BI-Tg (30/83 genes) than in PBF-Tg thyrocytes (12/83 genes). A differential p53 response was further evident following gamma-irradiation of cells, with fewer significant mRNA changes occurring in PTTG1-Tg (0/10 genes; P=NS) and BI-Tg (4/10 genes; $P<0.05$) than in WT primary thyrocytes (10/10 genes; $P<0.01$). By comparison, irradiation of PBF-Tg thyrocytes gave the greatest reduction in mRNA levels (6/10 genes; $P<0.05$) for genes such as Chek1 (4.4-fold; $P<0.01$) and Rad51 (8.4-fold; $P<0.01$). We therefore examined potential associations between PBF and DNA repair genes in human thyroid tumours. Importantly, a significant correlation was apparent between PBF and Chek1 ($R=0.44$; $P<0.05$; N=22), Fancg ($R=0.78$; $P<0.001$; N=22) and Mutyh ($R=0.62$; $P<0.05$; N=22). Together our data reveal for the first time that PBF and PTTG1 mediate distinct p53 response profiles in vivo. These results offer important insights for understanding the impact of proto-oncogenes on thyroid tumorigenesis and for identifying new tumour biomarkers.

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P150

Follow up of differentiated thyroid cancer survivors during pregnancy: a retrospective analysis

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Differentiated thyroid cancer (DTC) is common in female patients of reproductive age and generally has a good prognosis and so many patients may become