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

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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 intratumoral 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 (17ßHSD) 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, 17ßHSD7, and 17ßHSD2 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). 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.

Detection was also lost with a PTG-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 PTG protein expression was evident both in the cytoplasm and nucleus. In contrast, expression of T60-phosphorylated PTG was predominantly nuclear. As interaction with its binding partner PBF facilitates PTG nuclear localisation, and both proto-oncogenes review p53 stability and function, we assessed the relative contributions of PTG and PBF to p53 stability. Preliminary experiments demonstrated that transfection of wild-type PBF or PTG into HPV-positive 93-VU-147T HS SCC 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 PTG. Interestingly, transfection with a PBF mutant incapable of PTG interaction, or a PTG 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 PTG and PBF in modulation of p53 stability in head and neck cancers. Furthermore, PTG is phosphorylated at residue T60 in head and neck tumours, which may alter its well described mitotic regulatory function.