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Inhibition of tumorigenesis by peroxisome proliferator-activated receptor (PPAR)-dependent cell cycle blocks in human skin carcinoma cells

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ABSTRACT

To examine the functional role of peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) and PPARγ in skin cancer, stable cell lines were created in the A431 human squamous cell carcinoma cell line. Expression of PPAR target genes was greatly enhanced in response to ligand activation of PPARβ/δ or PPARγ in A431 cells expressing these receptors. PPARβ/δ expression blocked the cell cycle at the G2/M phase, and this effect was increased by ligand activation. Ligand activation of PPARβ/δ markedly inhibited clonogenicity as compared to vehicle-treated controls. Similarly, ligand activation of PPARγ in A431 cells expressing PPARγ resulted in reduced clonogenicity. Expression of either PPARβ/δ or PPARγ markedly reduced tumor volume in ectopic xenografts, while ligand activation of these receptors had little further influence on tumor volume. Collectively, these studies demonstrate that stable expression and activation of PPARβ/δ or PPARγ in A431 cells led to reduced tumorigenicity. Importantly, PPAR expression or ligand activation had major impacts on clonogenicity and/or tumor volume. Thus, PPARβ/δ or PPARγ could be therapeutically targeted for the treatment of squamous cell carcinomas.

1. Introduction

Targeting peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) or PPARγ has potential for the prevention and treatment of skin cancer because these transcription factors are nodal in nature and target multiple signaling pathways (reviewed in (Peters et al., 2015, 2012)). The first observation to suggest that PPARβ/δ could prevent chemically-induced skin cancer was the enhanced tumorigenicity found in *Pparβ/δ*-null mice as compared to controls (Kim et al., 2004). This was later supported by numerous studies showing that ligand activation of PPARβ/δ inhibits chemically-induced non-melanoma skin cancer through mechanisms that include induction of terminal differentiation, inhibition of mitosis, and promoting oncogene-induced senescence by modulating extracellular signal-regulated kinase (ERK) and protein kinase B (AKT) activities, and repressing endoplasmic reticulum stress (Bility et al., 2008; Bility et al., 2010; Zhu et al., 2011; Zhu et al., 2014a,b; Zhu et al., 2012). These mechanisms were verified using both in vivo and in vitro mouse models. Interestingly, the role of PPARβ/δ in other forms of cancer is less clear due to conflicting reports in the literature and may be due to differences in the models examined (reviewed in (Muller, 2017; Peters et al., 2015, 2012)). Thus, clinical studies examining the role of PPARβ/δ ligands in cancer prevention and therapy are lacking due to disparate results in rodent cancer and cell culture studies showing both anti- and pro-carcinogenic effects. This illustrates the need for more experimentation using alternative approaches to help resolve the role of PPARβ/δ in carcinogenesis.

Interestingly, it was also initially suggested that ligand activation of PPARγ promoted colon cancer, but subsequent studies showed that activation of PPARγ inhibited colon cancer, as well as other types of cancer (reviewed in (Peters et al., 2015, 2012)). This has led to clinical trials examining the efficacy of PPARγ ligands as cancer chemopreventive or chemotherapeutic agents in humans (reviewed in (Peters et al., 2012)). With respect to non-melanoma skin cancer, it was originally shown that mice with reduced expression and activity of PPARγ

Abbreviations: ADRP, adipocyte differentiation-related protein; ANGPTL4, angiopoietin-like protein 4; DMEM, Dulbecco's minimal essential medium; DMSO, dimethylsulfoxide; eGFP, enhanced green fluorescent protein; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IRES, internal ribosome entry site; PPAR, peroxisome proliferator-activated receptor; qPCR, quantitative real-time polymerase chain reaction

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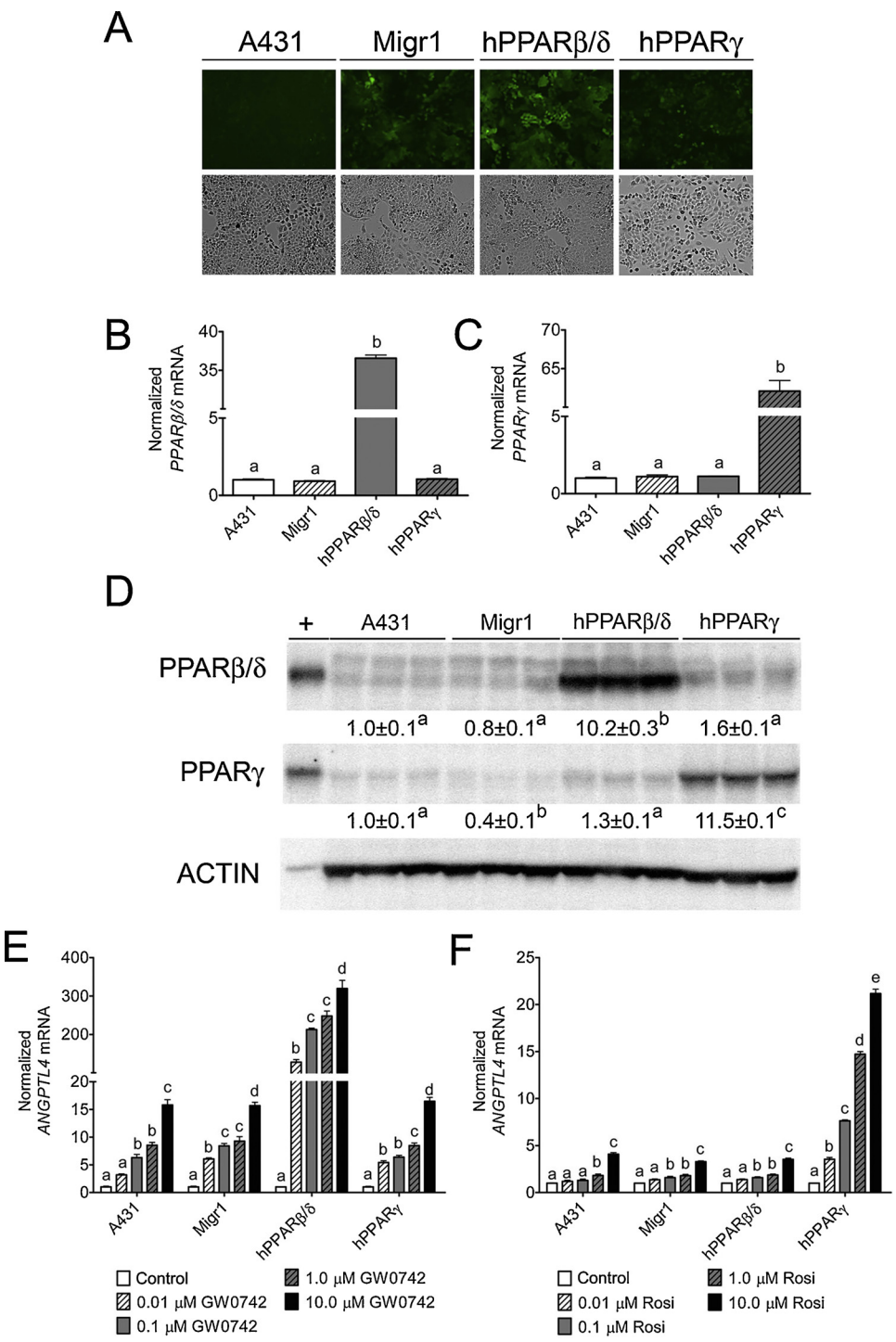

Fig. 1. Characterization of a human squamous cell carcinoma cell line (A431) over-expressing human PPARβ/δ or PPARγ. (A) Representative photomicrographs of A431 cells, A431-Migr1 control cells (Migr1), A431-Migr1-hPPARβ/δ cells (hPPARβ/δ), and A431-Migr1-hPPARγ cells (hPPARγ) examined by fluorescent microscopy (upper panels) or light microscopy (lower panels). qPCR analysis for mRNA expression of (B) PPARβ/δ or (C) PPARγ in the A431 cell lines, normalized to *GAPDH* mRNA. (D) Western blot analysis of PPARβ/δ or PPARγ in the A431 cell lines, normalized to ACTIN expression. + positive control: cell lysate from COS-1 cells transfected with hPPARβ/δ or hPPARγ expression vector. qPCR analysis of *ANGPTL4* mRNA in response to (E) the PPARβ/δ ligand GW0742 for 8 h or (F) the PPARγ ligand rosiglitazone (Rosi) for 24 h, normalized to the *GAPDH* mRNA. Data represents triplicate independent sample means ± S.E.M. Values with different letters are significantly different ($p \leq 0.05$).

exhibited enhanced sensitivity to chemically-induced skin cancer as compared to controls (Nicol et al., 2004); a phenotype similar to that observed of *Pparβ/δ*-null mice (Kim et al., 2004). Dietary or topical administration of two different PPARγ ligands (rosiglitazone or troglitazone) did not inhibit chemically-induced or ultraviolet (UV)-induced skin cancer, although dietary administration of troglitazone inhibited basal keratinocyte proliferation (He et al., 2005). A retrospective study in humans suggested that therapeutic use of the PPARγ ligand rosiglitazone may reduce the risk of non-melanoma skin cancer (Tseng, 2015). While clinical trials examining the effect of PPARγ ligands for cancer chemoprevention or chemotherapy are ongoing, similar to PPARβ/δ, there remain studies suggesting that PPARγ ligands may promote some, but not all, cancers (reviewed in (Peters et al., 2012)). This also illustrates the need for more experimentation using alternative approaches to help resolve the role of PPARγ in carcinogenesis.

One major variable that has led to much of the disparities with respect to the roles of PPARβ/δ and PPARγ in human cancer centers on relative expression of these proteins in tumor cells compared to normal control tissue. For example, it was originally reported that expression of PPARγ or PPARβ/δ were elevated in epithelial tumor cells as compared to non-transformed tissue (DuBois et al., 1998; He et al., 1999), but subsequent, more quantitative studies showed that relative expression of both PPARγ or PPARβ/δ is actually lower in epithelial tumors as compared to non-transformed tissue (Foreman et al., 2011; Modica