P-19 Diversification of Activation Mechanism of the Human Cyclooxynase-2 via a Promiscuous DR1 in the Promoter: Utilization of DR1 by Retinoic Acid Receptors

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Background & Objectives: Cyclooxygenase-2 (COX-2), a rate-limiting enzyme in prostaglandin (PG) biosynthetic pathway, is implicated in many pathophysiological processes. PGs produced via COX-2 are crucial in reproductive events in ovary and uterus. The human COX-2 (hCOX-2) promoter has a direct repeat 1 (DR1) previously shown to mediate PPAR responsiveness. During examination of PPAR delta responsiveness of this DR1 in uterine cells, we found that PPAR delta actually negated 9-cis-retinoic acid (9cRA)-induced promoter activity of the hCOX-2, rather than activating. Thus, we further characterized this DR1's activity and role in regulating hCOX-2 expression in AN3CA human uterine adenocarcinoma cells and F9 mouse embryonal carcinoma cells.

Method: The 7-kb human COX-2 promoter or the hCOX2/DR1 site was cloned into appropriate pGL3 luciferase vectors for reporter assays. Luciferase assays were performed in AN3CA and F9 cells. To examine direct binding of nuclear hormone receptors to hCOX2/DR1 site, EMSA was performed.

Results: In AN3CA cells, this DR1 mediates responsiveness to trans-retinoic acid (tRA) or 9cRA, but this effect was significantly suppressed by addition of PPAR delta. Truncated PPAR delta (tPPAR delta) lacking AF2 activation domain was incapable of suppressing RA-induced hCOX-2 DR1 activation, suggesting that cofactor recruitment mediated by AF2 is a key to suppression by PPAR delta. In F9 cells which abundantly express retinoic acid receptors (RARs), tRA and 9cRA greatly activated hCOX-2/DR1 responsiveness, and again, PPAR delta effectively repressed this phenomenon. EMSA showed that PPAR/RXR, as well as RAR beta/RXR and RXR/RXR were all capable of binding to hCOX-2 DR1, suggesting promiscuity of receptor binding on hCOX-2 DR1. Thus, antagonism between RAR and PPAR (especially PPAR delta) may be via competition to occupy available RXR partner and/or cofactors. We previously showed that three corepressors, RIP140, SMRT, and NCoR, all functions as transcriptional corepressors for PPAR delta. Thus, we tested if knockdown of a corepressor reverses negative action of PPAR delta on RA-induced activation of the hCOX-2/DR1. Our results show that knockdown of RIP140 via RNA interference had partial effect of ~30% reversal when added with full-length PPAR delta. But this was not observed in the case of truncated PPAR delta, suggesting a role for AF2 domain in mediating repressive effects. In the mouse uterus, PPAR delta is responsible to mediate COX-2 generated PGI2 signaling for successful implantation. In situ hybridization revealed that expression of all three subtypes of RAR was suppressed in the subepithelial stroma where the blastocyst makes initial contact for implantation, while PPAR delta is