SHORT COMMUNICATION

Expression of PGF$_{2\alpha}$ receptor mRNA in normal, hyperplastic and neoplastic skin

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Reverse transcription polymerase chain reaction (RT–PCR) and Northern blot analysis was used to determine the level of expression of prostaglandin F$_{2\alpha}$ (FP) receptor mRNA in various mouse tissues, including normal, hyperplastic and neoplastic mouse epidermis. Steady-state concentrations of FP receptor mRNA were low in normal and hyperplastic epidermis. The response of the epidermis to the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) was biphasic in that FP receptor mRNA was increased immediately after treatment, followed by a long-lasting down-regulation at later time points. FP receptor mRNA was down-regulated in the majority of papillomas obtained by the mouse skin carcinogenesis initiation–promotion protocol. In carcinomas, FP receptor mRNA expression was similar to that in normal epidermis. The steady-state concentration of FP mRNA was inversely correlated with PGF$_{2\alpha}$ levels in normal and hyperplastic epidermis and in papillomas, indicating that FP mRNA expression is regulated by this eicosanoid.

Non-steroidal anti-inflammatory drugs (NSAID), such as aspirin, sulindac, indomethacin, ibuprofen, etc., have been shown to inhibit experimental carcinogenesis in a variety of organs (for review, see ref. 1). According to epidemiological and clinical studies, NSAIDs significantly reduced colorectal tumor development in men (for review, see ref. 2). All NSAIDs suppress prostaglandin biosynthesis by inhibiting cyclooxygenases, i.e. enzymes catalyzing an essential step in prostanooid formation. In fact, prostaglandins were found to be dramatically elevated in many human and experimental tumors, and prevention of tumor development by NSAID was attributed to inhibition of prostaglandin production (1,2). Experimental mouse skin carcinogenesis so far provides the only model in which prostaglandins have been unequivocally shown to act as mediators of regenerative processes and tumorogenesis (3,4). Papillomas generated by the initiation–promotion protocol [using dimethylbenz(a)anthracene (DMBA) as an initiator and phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter] accumulate large amounts of prostaglandins E$_2$ (PGE$_2$) and PGF$_{2\alpha}$ (5). Moreover, the potent anti-promoting activity of the NSAID indomethacin could be specifically reversed by PGF$_{2\alpha}$ (6). In addition, when applied concomitantly with TPA, only this prostaglandin type exerted a co-promoting activity increasing the tumor response beyond that obtained by TPA alone. No information is yet available on the molecular mechanisms involved in the tumor-promoting activity of PGF$_{2\alpha}$. One obvious pathway through which PGF$_{2\alpha}$ may act is the interaction with its receptor expressed at the cell surface. A PGF$_{2\alpha}$ (FP)-specific receptor has recently been cloned from cDNA libraries originating from different species, including mouse (7). In this paper, we have analyzed the expression of this FP receptor in normal, hyperplastic and neoplastic mouse epidermis, as well as in various other mouse tissues.

Seven-week-old female NMRI mice (BRL, Füllinsdorf, Switzerland) were used in the animal experiments. Shaving of the back skin with electrical clippers was performed 3 days prior to treatment. For topical applications, compounds were dissolved in 0.1 ml acetone and applied onto the shaved back skin. Mice were killed at varying time-points, the back skin was dissected and snap-frozen at –70°C using a cold table. Mouse skin tumors were generated by the mouse skin carcinogenesis initiation–promotion protocol. For topical applications, compounds were dissolved in 0.1 ml acetone; twice-weekly applications for 20 weeks). The steady-state concentration of FP receptor mRNA was diseregulated by this eicosanoid.
Fig. 1. RT–PCR of FP receptor mRNA from various mouse tissues. RNA from each sample was reverse-transcribed to cDNA and PCRs were run with a specific primer set for FP receptor mRNA (upper panel) and with a primer set for β-actin as an internal control (lower panel), as described in the text. Lanes 1, footsole; 2, trachea; 3, lung; 4, tongue; 5, forestomach; 6, brain; 7, thymocytes; 8, reticulocytes; 9, colon; 10, intestine; 11, liver; 12, testis; 13, skeletal muscle; 14, H2O (no cDNA template); 15, kidney (positive control); 16, marker.

Fig. 2. RT–PCR of FP receptor mRNA from normal and TPA-treated mouse epidermis. After epicutaneous application of acetone (0.1 ml) or 10 nmol TPA (dissolved in 0.1 ml acetone), total RNA was extracted from epidermis at the times indicated and reverse transcribed to cDNA. PCRs were run with two FP-receptor-specific primer sets (upper panel) and with a primer set for β-actin as internal control (lower panel). Lane 1, acetone-treated epidermis (30 min); 2, epidermis 30 min after TPA treatment; 3, 1 h after TPA treatment; 4, 2 h after TPA treatment; 5, 4 h after TPA treatment; 6, 6 h after TPA treatment; 7, 24 h after TPA treatment; 8, 48 h after TPA treatment; 9, H2O (no cDNA template); 10, kidney (positive control); 11, marker.

592 bp was obtained, and with the forward primer 5′-GCTCTTTGTTTCTTCTCCTC-3′ and reverse primer 5′-TGCTTGGCTGCTTCTCCT-3′, a PCR product of 446 bp was obtained. In order to discriminate the amplification products from those originating from contaminating genomic DNA, the primer sets were designed to flank intron 2, which is more than 7.5 kb in size. Amplification of a β-actin DNA fragment (429 bp) was also performed as an internal control using the forward primer 5′-AAACTGGAACGGTGACAAC-3′ and the reverse primer 5′-GCTGCTCCTAACACCTCACC-3′. The PCR reactions were primed with 1 µl of the cDNA reactions using 20 pmol primers in 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 0.2 mg/ml bovine serum albumin with 0.2 mM of each dNTP and 0.5 µl Taq polymerase (5 U/µl; Appligene Oncor, Illkirch, France) in 50 µl reactions. The PCR was programmed in a PTC-200 DNA Engine (MJ Research, Watertown, USA). The amplification cycle for the FP receptor fragment consisted of an initial denaturation at 94°C for 5 min, followed by 94°C for 1 min, 60°C (fragment of 446 bp) or 55°C (fragment of 592 bp) for 1 min, 72°C for 1 min and 30 cycles, and termination at 72°C for 10 min. From each PCR reaction, a 24 µl aliquot was analyzed by electrophoresis on a 1.4% agarose gel. The amplification cycle for the β-actin fragment consisted of an initial denaturation at 95°C for 5 min, followed by 94°C for 90 s, 54°C for 90 s, 72°C for 90 s for 30 cycles, and termination at 72°C for 10 min. From each β-actin PCR reaction, an 8 µl aliquot was analyzed by electrophoresis on a 1.4% agarose gel. The identity of the PCR products was confirmed by sequence determination using an ABI Big Dye Terminator Cycle Sequencing Ready Reaction kit and the products were resolved on an ABI Prism 310 Genetic Analyzer (Perkin-Elmer/Applied
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Biosystems, Weiterstadt, Germany). The sequences were assembled and analyzed using the Heidelberg Unix Sequence Analysis Resources (HUSAR) software programs.

Northern gels loaded with 10–14 µg RNA were electrophoresed and RNA was transferred to Hybond-N$^+$ membranes (Amersham/Pharmacia, Freiburg, Germany) by established procedures (8). Labeling was performed using the Megaprime DNA labeling kit (Amersham/Pharmacia, Freiburg, Germany) with gel electrophoresed and purified cDNA fragments. The filters were washed with a final stringency of 0.1× standard saline citrate, 0.5% sodium docecyl sulfate for 20 min and exposed to film using intensifying screens at –80°C. Standardization of RNA loading was performed by rehybridization of the blots with a 18S-rRNA-specific probe.

Using murine FP receptor cDNA sequence information (7), two pairs of oligonucleotide primers were designed, yielding FP receptor-specific amplification products of 592 and 446 bp, as confirmed by DNA sequencing. RT–PCR analysis was used to measure the expression of FP receptor mRNA in murine tissues, including footsole, forestomach, tongue and trachea, colon, kidney, liver and skeletal muscle, as well as in brain. No FP receptor mRNA expression was detected in testis, thombocytes or reticulocytes (Figure 1). These results confirm previous data showing that uterine tissue and kidney exhibited the highest level of FP receptor mRNA expression (7). Expression in footsole, forestomach, tongue and trachea is reported here for the first time. In contrast to a previous study (7), FP receptor mRNA was also detected in brain, liver and intestines.

Using two different specific primer sets, FP receptor mRNA was also found in murine skin epidermis (Figure 2). Upon epicutaneous administration of the phorbol ester TPA, a rapid increase of the FP receptor mRNA content was observed, which fell back to control levels around 2–4 h after treatment, and later on dropped below the control, to remain suppressed for up to 48 h after treatment (Figure 2). The transient increase of FP receptor mRNA expression induced by TPA was confirmed by northern blot analyses (Figure 3). As shown in Figure 4, FP receptor mRNA was also detected in hyperplastic epidermis obtained upon exposure of adult mice skin to chronic TPA treatment (9), and in neonatal mouse skin, which constitutively exhibits a hyperplastic phenotype (10).

In papillomas obtained by the initiation–promotion protocol, the steady-state concentration of FP receptor mRNA was found to be moderately or strongly reduced (Figures 3 and 5). Treatment of papilloma-bearing animals with TPA, however, induced a transient increase of FP receptor mRNA. In contrast to papillomas, carcinomas exhibited a FP receptor mRNA content which was only slightly reduced when compared with normal skin (Figure 5). These data show the FP receptor mRNA to be constitutively expressed at low levels in murine epidermis, indicating that keratinocytes not only generate PGF$_{2\alpha}$ (6), but are also effector cells for this eicosanoid. This conclusion was confirmed by the observation that keratinocytes in culture were also found to express FP receptor mRNA (data not shown). A rapid induction of FP receptor mRNA expression by TPA has also been reported for corpora lutea granulosa cells, which show a similar time course (11). A unique feature of skin epidermis is the down-regulation of FP receptor mRNA seen upon prolonged TPA treatment. This drop in expression of FP receptor mRNA, which is inversely correlated with TPA-induced synthesis of PGF$_{2\alpha}$ (5,6), indicates an agonist-induced down-regulation of the receptor mRNA. Such an effect of PGF$_{2\alpha}$ has indeed been reported for ovine corpora lutea (12). Conversely, suppression of prostaglandin biosynthesis led to an induction of FP receptor mRNA expression in blood vessels and brain synaptosomes of the newborn pig. This effect was specifically counteracted by PGF$_{2\alpha}$ or FP receptor agonists (13,14). Therefore, the high level of PGF$_{2\alpha}$ in papillomas (5) may be responsible for the down-regulation of FP receptor mRNA in these tumors. On the other hand, FP receptor mRNA expression was found to be at similar levels, rather than reduced, in chronic hyperplastic skin as compared with normal epidermis. This observation is in agreement with a low PGF$_{2\alpha}$ level both in neonatal and chronically TPA-treated hyperplastic skin epidermis (5). It remains to be established, however, whether direct treatment of mouse skin with NSAID or PGF$_{2\alpha}$ leads to changes in FP receptor mRNA expression and whether such changes are reflected at the level of receptor protein or whether they influence receptor activity.

As far as the co-promoting effect of PGF$_{2\alpha}$ is concerned, it is still unclear whether a down-regulation of FP receptor expression in papillomas provides a selective advantage for initiated cells, e.g. through protection against cell death. In support of this, a stimulatory effect of PGF$_{2\alpha}$ on programmed cell death might, for instance, be postulated. Such an effect may indeed be involved in the degradation of corpus luteum cells (15), whereas for keratinocytes this still remains to be shown.

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References


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