Expression of the transmembrane protein tyrosine phosphatase RPTPα in human oral squamous cell carcinoma

Abstract  Little is known about the role of protein-tyrosine phosphatases (PTPs), the cellular counterparts of protein-tyrosine kinases, both for normal growth regulation and for its dysregulation in cancer. The receptor-like PTPα (RPTPα) may play a positive role in growth regulation and has been shown to be overexpressed in colon carcinoma. An RNA/RNA in situ hybridisation protocol for RPTPα as well as RPTPα immunohistochemistry was developed to evaluate RPTPα expression in oral squamous cell carcinomas (OSCCs) of different histological grade and to reveal the synthetically active cells and their tissue distribution. In well-differentiated OSCC (G1), RPTPα mRNA could be detected by in situ hybridisation exclusively in stroma cells (fibro/myofibroblasts and inflammatory cells). A higher histological grade (G2/G3) was associated with an increased number of RPTPα-synthesising carcinoma cells haphazardly distributed within invading tumour areas. Consistent results were obtained by immunocytochemistry. Thus, both carcinoma dedifferentiation and stroma recruitment and activation seem to be associated with an upregulation of RPTPα expression in OSCC. The results speak in favour of the important role of activation of stroma fibro/myofibroblasts influencing the biological behaviour of epithelial tumours and also suggest that elevated RPTPα expression may be a more general marker for proliferating or dedifferentiated cells.

Introduction

Protein-tyrosine phosphorylation is an important cellular mechanism for the regulation of growth and differentiation, and aberrant tyrosine phosphorylation has been linked to various diseases including human malignancies. Whereas the significance of different protein-tyrosine kinases (PTKs) has been studied in great detail, much less is known about the role of their counterparts, protein-tyrosine phosphatases (PTPs), both for normal growth regulation and for dysregulation in cancer (Hunter 1995, 1998). Numerous PTPs have been identified over the last 10 years falling into two main classes, the cytosolic and the transmembrane, receptor-like PTPs (Neel and Tonks 1997; Schaapveld et al. 1997).

The receptor-like PTPα (RPTPα) is a widely expressed enzyme with particularly high levels in brain and kidney (Matthews et al. 1990; Sap et al. 1990). It consists of a short, heavily glycosylated extracellular domain, a single transmembrane domain and a cytosolic domain with two intracellular catalytic PTP domains, D1 and D2. A higher histological grade (G2/G3) was associated with an increased number of RPTPα-synthesising carcinoma cells haphazardly distributed within invading tumour areas. Consistent results were obtained by immunocytochemistry. Thus, both carcinoma dedifferentiation and stroma recruitment and activation seem to be associated with an upregulation of RPTPα expression in OSCC. The results speak in favour of the important role of activation of stroma fibro/myofibroblasts influencing the biological behaviour of epithelial tumours and also suggest that elevated RPTPα expression may be a more general marker for proliferating or dedifferentiated cells.
1995; Lammers et al. 1997; Jacob et al. 1998). In transient overexpression systems, RPTPα also potently dephosphorylates the receptor for platelet-derived growth factor (PDGF; S. Groß, X. Luo and F. D. Böhmer, unpublished data). Thus, depending on the cellular context, RPTPα expression and activity level may have positive or negative consequences for cell growth and differentiation.

Due to this implication of RPTPα in cell growth regulation and possible transformation, its tissue distribution in carcinoma may contribute to the understanding of carcinoma invasion and progression. Therefore, an RNA/RNA in situ hybridisation protocol for RPTPα has been developed to reveal the synthetically active cells and their tissue distribution in tumours. Analysis of oral squamous cell carcinoma (OSCC) with this technique revealed RPTPα expression and cellular distribution in relation to the histological grade.

Materials and methods

Tissue material

The RPTPα mRNA in situ hybridisation and the corresponding immunohistochemistry were performed on 12 OSCCs. Three carcinomas were well differentiated (G1), 6 were moderately differentiated (G2) and 3 were less differentiated (G3). Tissue specimens included areas of non-neoplastic, hyperplastic and dysplastic epithelia. Blocks of 4×4×4 mm of fresh, surgically obtained tissue were immediately shock frozen in isopropanol cooled by liquid nitrogen and stored at -75°C. The diagnosis was confirmed in the corresponding paraffin-embedded tissue according to the WHO classification criteria (Pindborg et al. 1997). Histological grading of malignancy was performed using the method reported by Bryne and coworkers (1992).

In situ hybridisation

A cDNA for murine RPTPα (LRP) was kindly provided by Dr. M. Thomas (Washington). A 728-bp fragment (nucleotides 1–728 of LRP cDNA; Matthews et al. 1990) covering the coding sequence for the extracellular, transmembrane and juxtamembrane domain was obtained by EcoRI digestion and inserted into EcoRI-digested pBluescriptIIKS+ vector (Stratagene, La Jolla, USA). Sense or antisense RNA probes were generated by in vitro transcription of the HindIII or BamHI linearised plasmid using digoxigenin-labelled uridine triphosphate as substrate (DIG RNA labelling kit; Boehringer Mannheim, Mannheim, Germany) and T7 or T3 RNA polymerase. Unincorporated nucleotides were removed by ethanol precipitation. The precipitate was dissolved in 100 µl diethyl pyrocarbonate-treated and RNase inhibitor-containing water. The transcripts were analysed by agarose gel electrophoresis and dot blot assay.

In situ hybridisation was carried as described previously (Berndt et al. 1998). Sections, 7–10 µm thick, of immediately snap-frozen tissue were heated for 2 min at 50°C to fix the RNA in the tissue. The sections were allowed to air dry for 30 min and were then fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) for 20 min at 4°C. After washing in PBS (1× 5 min) and 2×SSC (2×5 min), the prehybridisation solution was applied on each slide for 60 min at 37°C. The prehybridisation solution contained 4×SSC, 10% dextran sulphate, 1×Denhardt’s solution, 2 mM EDTA, 50% deionised formamide, 100 µg/ml herring sperm DNA and 100 µg/ml tRNA. After removal of this solution, each section was covered with 100 µl prehybridisation solution containing 200–1000 ng/ml of DIG-labelled antisense cRNA probe and incubated at 37°C overnight.

As hybridisation controls, the antisense cRNA probe was replaced by RPTPα sense cRNA or was completely omitted from the hybridisation to evaluate the quality of the colour-detection system. After hybridisation, unbound probe was washed from the sections as follows: 1×5 min with 2×SSC at 37°C, 3×5 min with 60% formamide in 0.2×SSC at 37°C and 2×5 min with 2×SSC at room temperature.

Hybridised DIG-labelled cRNA probes were detected using the components of the DIG nucleic acid detection kit (Boehringer Mannheim) following the protocol recommended by the manufacturer. The colour reaction was carried out for up to 24 h at room temperature.

Immunohistochemistry

Cryostat sections of the respective frozen tissue samples were fixed in ice-cold acetone for 15 min and subjected to immunohistochemistry. As primary anti-RPTPα antibody, the clone 21 (Transduction Laboratories, Lexington, USA) was used. This antibody detects a single band of 140–150 kDa in immunoblotting with extracts of human U937 and HEK 293 cells (Fig. 1) or SKN human neuroblastoma cells (Transduction Laboratories, 1998 Catalogue). Immunohistochemical staining was performed using the alkaline phosphatase monoclonal anti-alkaline phosphatase (APAAP) method. The primary antibody was incubated with the tissue sections for 30 min at room temperature. After washing with TRIS-buffer, sections were treated with rabbit anti-mouse immunoglobulin (diluted 1:70; Dako, Glostrup, Denmark), and then with the mouse APAAP complex (Dako). Both incubations were done for 30 min at room temperature. To increase the staining intensity, the incubation with the rabbit anti-mouse immunoglobulin and with the APAAP complex was repeated twice. Naphthol AS-BI phosphate (Sigma, St. Louis, USA.) and new fuchsin (Merck, Darmstadt, Germany) were used as substrate and developer, respectively. To inhibit endogenous tissue enzyme activity, the developing solution was supplemented with 0.25 mmol/l levamisole (Sigma). To evaluate the specificity of immunostaining, the primary antibody was replaced by non-immune serum as a negative control.

Results

Analysis of synthesis and distribution of RPTPα: RPTPα immunohistochemistry

In normal adult and hyperplastic squamous epithelium, RPTPα was not detectable using the antibody 21. In the G1 and G2 OSCCs, RPTPα distribution could be demonstrated by immunohistochemistry to be restricted to