

P2X₅ and P2X₇ receptors in human warts and CIN 612 organotypic raft cultures of human papillomavirus infected keratinocytes

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Figures 1, 2 and 3 were printed in black and white. They should have been printed in colour as shown.

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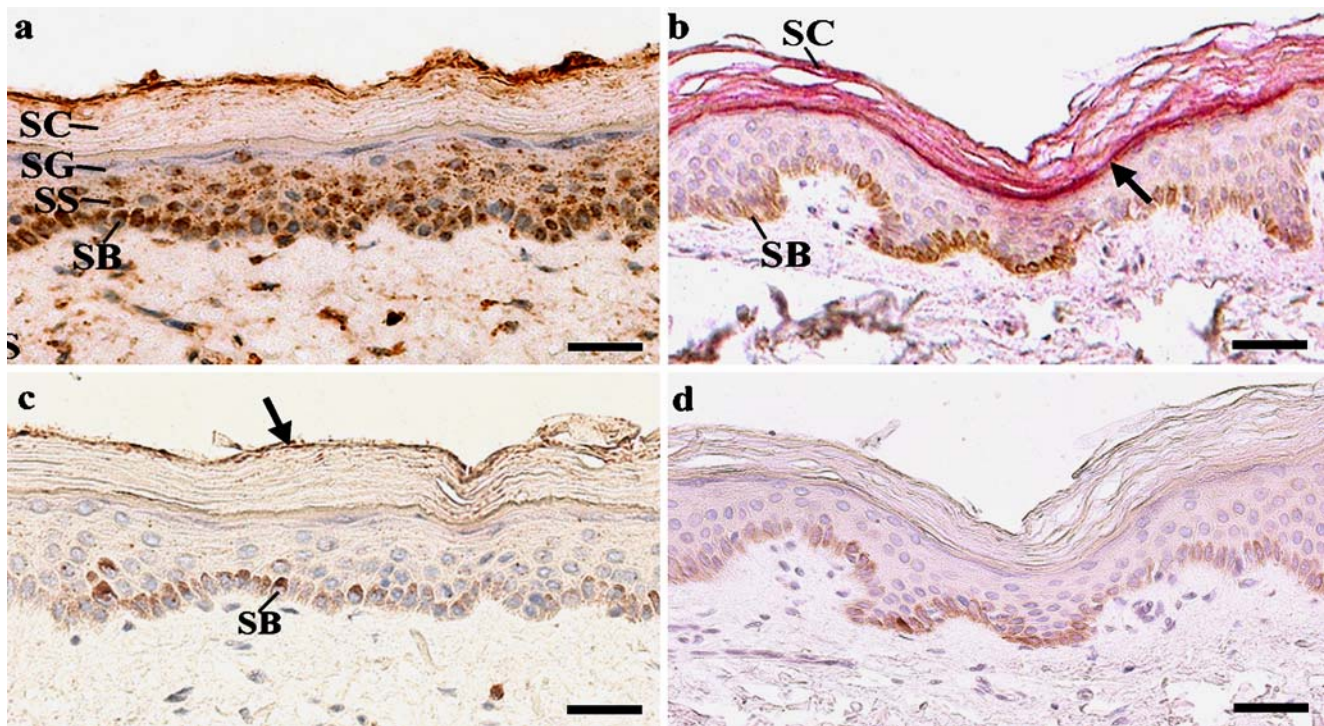
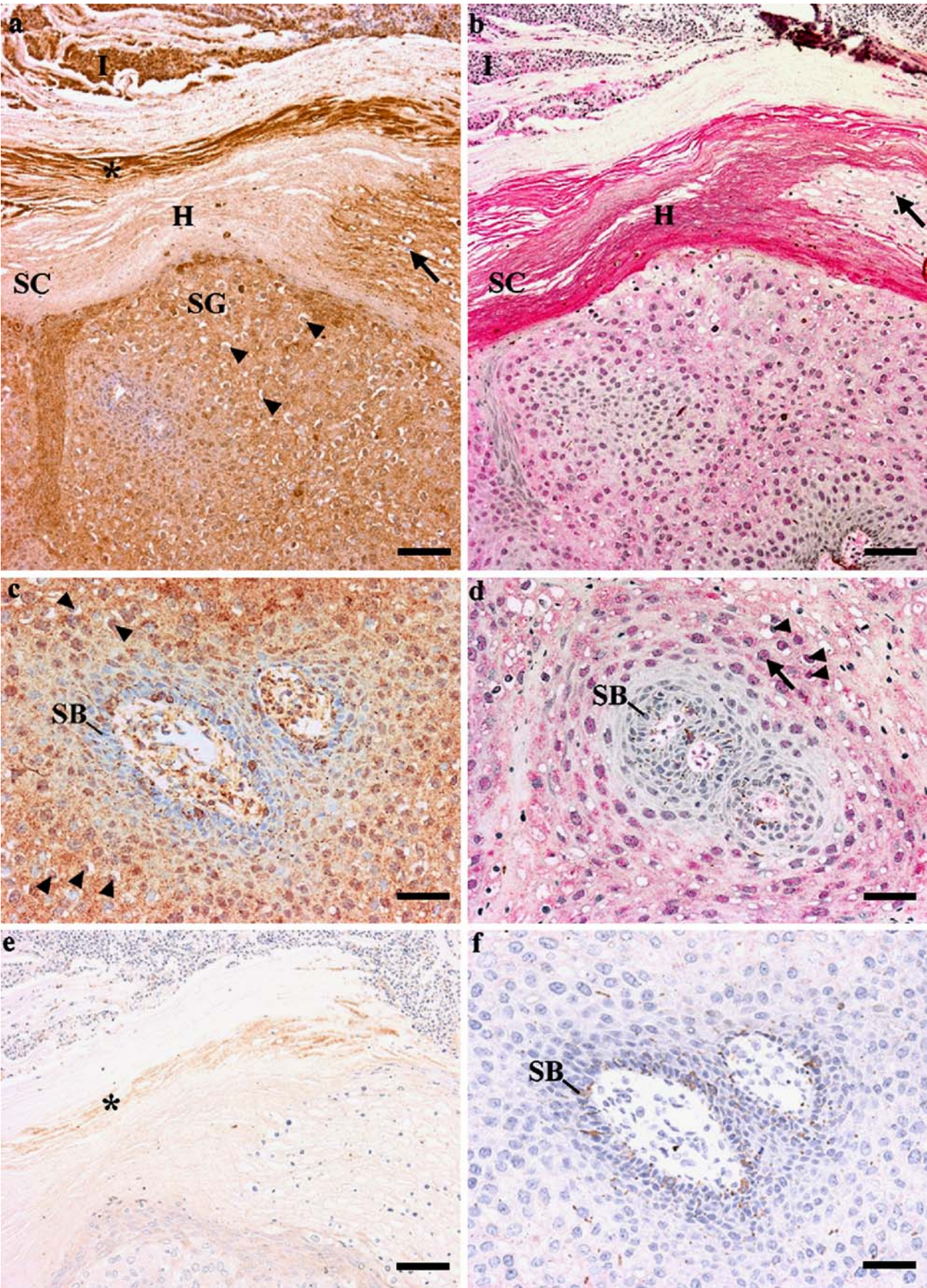


Figure 1 Expression of P2X₅ and P2X₇ receptors in paraffin sections of normal human skin. Nuclei were counterstained blue with haematoxylin. (a) In normal skin, P2X₅ immunoreactivity (brown) was found in basal keratinocytes (B) (although this was not easy to distinguish from melanin), and in the stratum spinosum (SS), and in very few cells in the stratum granulosum (SG). P2X₅ receptor staining was absent from the stratum corneum (SC), apart from at the outer edge. P2X₅ receptor staining was confined largely to the cell membranes in the basal layer, and found in the cytoplasm, and occasionally in the nucleus in both basal and suprabasal keratinocytes.

Scale bar, 25 µm. (b) P2X₇ immunoreactivity (pink) was present in the epidermis of all normal skin samples, and was associated with cells and cell fragments (arrow) in the stratum corneum (SC). Note the brown melanin in the basal layer (B). Scale bar, 25 µm. (c) There was residual staining of the outermost edge of the stratum corneum (arrow) with the P2X₅ receptor antibody no primary control, and therefore this was non-specific staining. Note the brown melanin in the basal layer (B). Scale bar, 25 µm. (d) There was no staining in the no primary control for the P2X₇ receptor antibody. Scale bar, 25 µm.

Figure 2 Expression of P2X₅ and P2X₇ receptors in paraffin sections of human warts. Nuclei were counterstained blue with haematoxylin. (a) Low power view of P2X₅ immunoreactivity (brown) in the wart. P2X₅ receptors were present within the keratinocytes of the wart. There was marked hyperkeratosis (H), which was negative for P2X₅ receptors, although areas of parakeratosis were positive (arrow). At the outer edge of the stratum corneum there was a band of heavy staining (asterisk). P2X₅ receptors were also found in the inflammatory cell infiltrate (I) above the stratum corneum (SC). There was a prominent granular layer (SG), within which cells (koilocytes) showed typical cytoplasmic vacuolation (arrowheads). Scale bar, 100 µm. (b) Low power view of P2X₇ immunoreactivity (pink) in the wart. P2X₇ receptors were strongly present within the hyperkeratotic (H) areas of the stratum corneum (SC), but not in areas of parakeratosis (arrow). P2X₇ receptors were weakly present in the wart keratinocytes, and mainly found in the nucleus. P2X₇ receptors were also weakly found

in the inflammatory cell infiltrate (I) above the stratum corneum. Scale bar, 100 µm. (c) High power view of P2X₅ immunoreactivity (brown) in wart keratinocytes. There were a few positive cells in the basal layer (B), but most of the positively stained cells were in the suprabasal layers. Koilocytes showed P2X₅ receptor staining in the nucleus (arrowheads). Scale bar, 50 µm. (d) P2X₇ immunoreactivity (pink) was present in the suprabasal layers of the wart, in either large, flat nuclei with an obvious nuclear membrane (arrow), or in koilocytes, where the receptor was prominent in shrunken, pyknotic nuclei, (arrowheads). P2X₇ receptors were not found in the basal layer (B) of the wart. Scale bar, 50 µm. (e) There was residual staining of the outermost edge of the stratum corneum (asterisk) with the P2X₅ receptor antibody no primary control, and therefore this was non-specific staining. Scale bar, 100 µm. (f) There was no staining in keratinocytes of the wart with the P2X₅ receptor antibody no primary control. There was some melanin in the basal layer (B) of the wart. Scale bar, 50 µm.



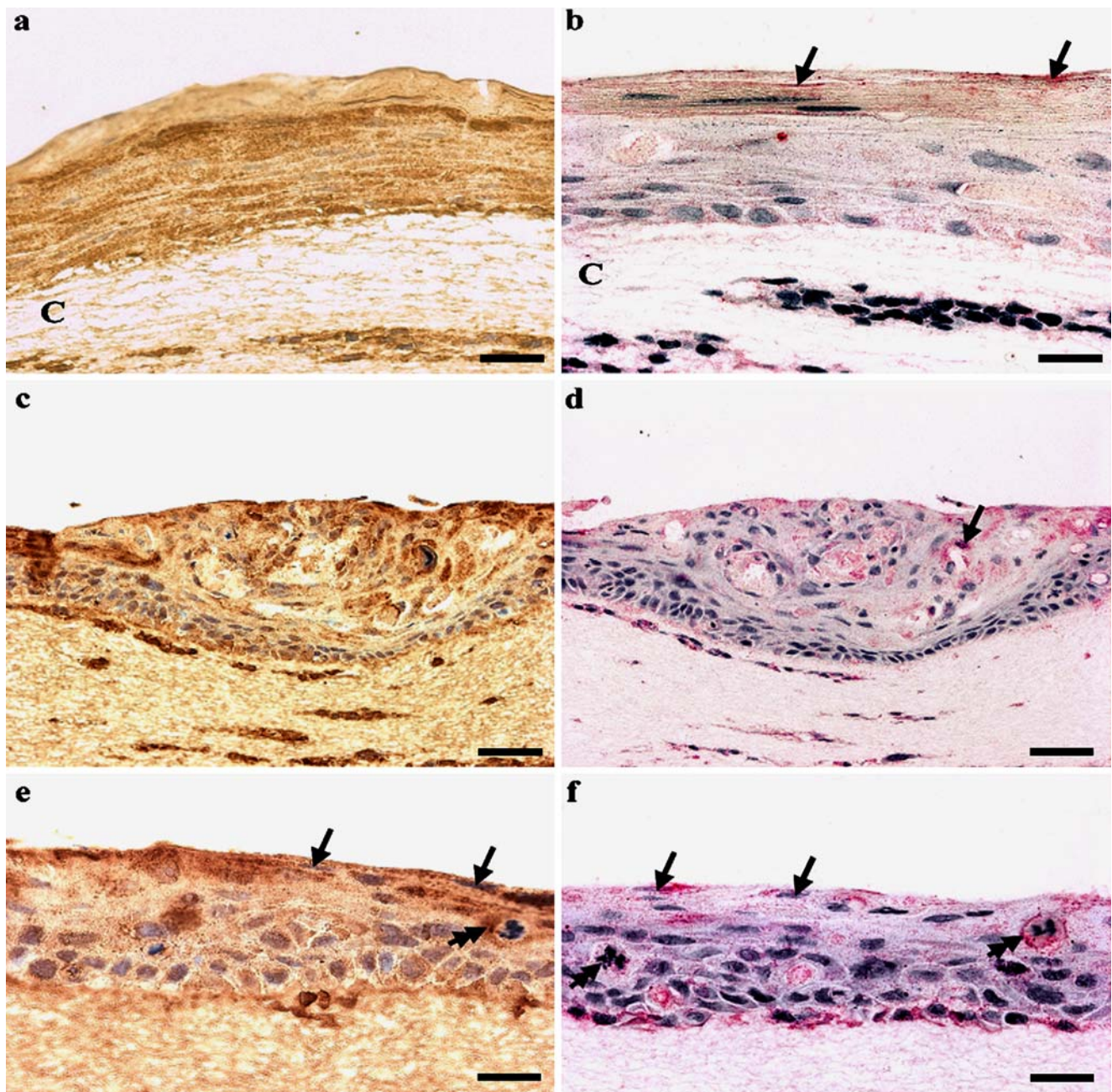


Figure 3 Expression of P2X₅ and P2X₇ receptors in paraffin sections of raft cultures of normal human keratinocytes and of CIN 612 (HPV 31) cells. Nuclei were counterstained blue with haematoxylin. (a) P2X₅ immunoreactivity (*brown*) was present throughout all layers of the raft cultures of normal human foreskin keratinocytes, where the staining was confined largely to the cell membranes and the cytoplasm. The raft culture was supported on a collagen matrix (C). Scale bar. 25 μm. (b) P2X₇ immunoreactivity (*pink*) was present in the raft cultures of normal human foreskin keratinocytes, staining weakly within the uppermost layer (*arrows*). Scale bar. 25 μm. (c) P2X₅

immunoreactivity(*brown*) was present in the CIN 612 (HPV 31) raft keratinocytes, staining all layers of the raft. Scale bar. 50 μm. (d) P2X₇ immunoreactivity (*pink*) was present in the CIN 612 raft and was associated with the cell cytoplasm and nucleus (*arrow*). Scale bar. 50 μm. (e, f) High power views of CIN 612 (HPV 31) raft cultures: the uppermost layers are highly disorganised, with nucleated cells at the surface of the raft (*arrows*). There was also positive staining in the cytoplasm of mitotic cells (double arrows) within the raft for both (e) P2X₅ receptors (*brown*) Scale bar 25 μm. and f P2X₇ receptors (*pink*). Scale bar. 25 μm.