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Letter to the Editor

DIRECTIONAL SPECIFICITY OF PROSTATE STROMAL TO EPITHELIAL CELL COMMUNICATION VIA FGF7/FGFR2 IS SET BY CELL- AND FGFR2 ISOFORM–SPECIFIC HEPARAN SULFATE

Dear Editor:

Communication between epithelial and stromal compartments of parenchymal organs is mediated, in part, by members of the fibroblast growth factor (FGF) family in which different isotypes of the FGF signal and the FGF tyrosine kinase receptor are partitioned between compartments (McKeehan et al., 1998). Similar to other parenchymal organs with both epithelial and stromal compartments, prostate epithelial cells express the IIIb splice variant of the type 2 FGF receptor (FGFR2), whereas stromal cells express the IIIc variant as well as FGFR1IIIc, which is not expressed in the epithelial cells (Yan et al., 1993; Feng et al., 1997). Expression of FGF7 and FGF10 within the current family of 23 FGF homologs occurs exclusively in the stromal cells (Yan et al., 1992; Lu et al., 1999). This partitioning of FGF7/FGF10 and FGFR2 isoforms is thought to underlie directionally specific signaling from stromal to epithelial cells. Epithelial cells respond only to FGF7/FGF10, but not to FGF2, whereas stromal cells do not respond to FGF7/FGF10, but respond to FGF2 (Yan et al., 1992; Lu et al., 1999). Recent reports suggest that the specificity of the FGFR isoforms for the FGF isoforms is mammalian cell–context dependent (Kan et al., 1999; Uematsu et al., 2000). In liver cells, FGFR4 recognizes specifically FGF1 whereas cell-free recombinant FGFR4 recognizes FGF1 and FGF2 equally. Conversely, FGFR4 recognizes only FGF2 in endothelial cells similar to the resident FGFR1 (Kan et al., 1999). The apparent loss of specificity of cell-free FGFR4 was traced to the use of heparin instead of cell-derived heparan sulfate in binary complexes with FGFR4 used to assess the binding of FGF1 and FGF2 to cell-free FGFR4 (Kan et al., 1999). Cell-type specificity for FGF1 or FGF2 was restored to the cell-free FGFR4 by substitution of the heparin with heparan sulfate from liver or endothelial cells, respectively (Kan et al., 1999). More surprising was the demonstration that cell-free binary complexes of heparin and FGFR2IIIb and FGFR2IIIc failed to exhibit selectivity for FGF7 or FGF2, respectively, in marked contrast to their profiles in epithelial and stromal cells (Uematsu et al., 2000). Here we show that, similar to FGFR4, the cell type selectivity of FGFR2IIIb and FGFR2IIIc for FGF is dependent on the cell membrane context and is determined by cell type–specific heparan sulfate.

Constructs coding for the two Ig module β isoforms of the ectodomains, transmembrane domains and part of the intracellular juxtamembrane domains of FGFR2IIIb, FGFR2IIIc, and FGFR1IIIc fused to glutathione-S-transferase on the intracellular domain, were expressed into the membranes of baculoviral-infected Sf9 insect cells as described (Uematsu et al., 2000). After extraction with detergent and immobilization on Glutathione (GSH)–agarose beads, binary complexes were prepared with heparin or cell-derived heparan sulfate, and the binding of radiolabeled FGF1, FGF2, and FGF7 was compared (Uematsu et al., 2000). Active cellular heparan sulfate proteoglycan (HSPG) was harvested from 40 plastic 75-cm² culture flasks of subconfluent Dunning R3327PAP tumor prostate epithelial cells (DTE) or stromal cells (DTS) by trypsin treatment as previously described (Kan et al., 1999). The HSPGs were partially purified by high-performance liquid chromatography (HPLC) using ion exchange (TSK-DEAE-5 PW, 75 × 7.5 mm; Bio-Rad, Richmond, CA), followed by molecular filtration (Bio-Sil SEC-400, 300 × 7.8 mm; Bio-Rad), and traced and quantitated as described (Kan et al., 1999; McKeehan et al., 1999; Wu et al., 2001). The indicated isoforms of immobilized FGFR were incubated with HSPG preparations from either DTE or DTS cultures to allow the FGFR to affinity select FGFR-specific fractions of HSPG by formation of a binary complex prior to introduction of radiolabeled FGF. Affinity purification of the DTE cell HSPG that interacts with Ig module II of FGFR1 to homogeneity, with respect to core protein sequence, indicates that structural domains within the ectodomain of FGFR interact with a rare species of cellular HSPG with respect to the heparan sulfate chain (Wu et al., 2001). Moreover, the presence of soluble-free heparin or HSPG in binding assays can have dramatic effect on the apparent specificity of FGF binding to FGFR by formation of heparin–FGF complexes that cannot interact with FGFR (Uematsu et al., 2000). Therefore, care was taken to remove unbound heparin or HSPG by washing the binary complexes extensively prior to introduction of radiolabeled FGF1, FGF2, or FGF7. High affinity binding to the binary complexes was assessed by covalent affinity crosslinking of the FGF to the FGFR part of the complex and autoradiography of radiolabeled complexes (Fig. 1).

As demonstrated previously (Uematsu et al., 2000), cell-free binary complexes of heparin (H) and FGFR2IIIb or FGFR2IIIc exhibited little specificity for FGF1, FGF2, or FGF7, whereas heparin–FGFR1IIIc complexes failed to bind FGF7 (Fig. 1). Both HSPGs derived from DTS and DTE cells supported binding of FGF1 to FGFR2IIIb, the resident isoform in epithelial cells. In contrast, HSPG from both DTS and DTE cells failed to support the binding of FGF2 to FGFR2IIIb. The HSPG from both cell types supported the binding of FGF7 to FGFR2IIIb. This suggests that the specificity of epithelial cells for FGF7, relative to FGF2, lies in FGFR2IIIb-specific HSPG that is not cell-specific. FGFR2IIIb is expressed ectopically in mesenchymal cells in two rare cases of Apert's syndrome which is thought to result from the abnormal autocrine activity resulting from activation by FGF7 or FGF10 in the stromal cells (Oldridge et al., 1999). Our results with DTS cells

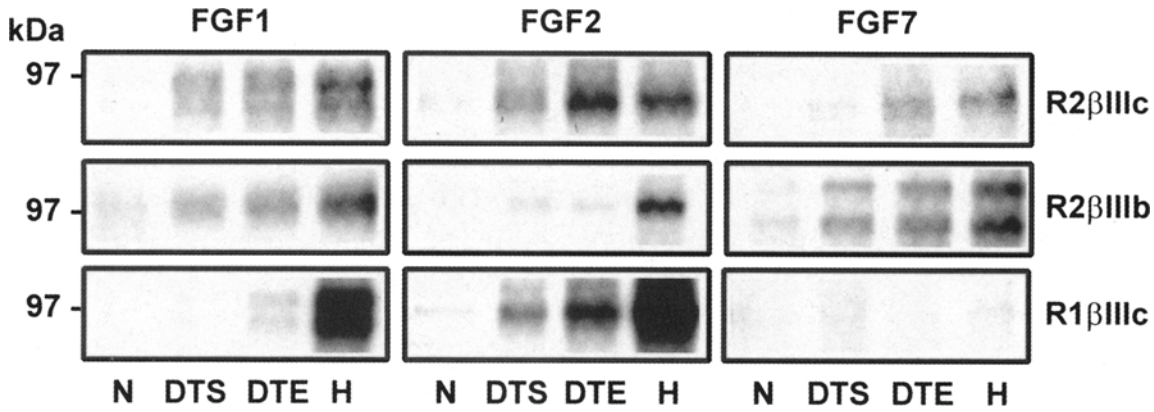

FIG. 1. Effect of heparin or cellular heparan sulfate on specificity of binary complexes with the FGFR isoforms for FGF. Heparin (H) (100 ng/ml) or cellular heparan sulfate (25 ng/ml) isolated from DTS or DTE cells as described in the text were first incubated with 250 μl of the indicated isoform of FGFR immobilized on GSH beads for 1 h at 4° C. Optimum conditions that promote the FGF-dependent conformation of FGFR and specific high-affinity interaction of heparin and heparan sulfate with the FGFR ectodomain were employed as described (Kan et al., 1996; Kan et al., 1999; McKeehan, et al., 1999). After extensive washing with phosphate-buffered saline containing 10 m*M* $MgCl_2$ to remove unbound heparin and heparan sulfate, the indicated ^{125}I-FGF was added, which had been previously standardized with respect to specific activity and biological activity. Covalent complexes were generated and isolated, then subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and autoradiography as described (Kan et al., 1991; 1996; 1999).

suggest that stromal cells exhibit an HSPG that would pair with ectopic FGFR2IIIb and support the autocrine activity of FGF7. Both DTS and DTE cell HSPGs support the binding of FGF1 and FGF2 to the FGFR2IIIc isoform (Fig. 1, *middle panel*). Expression of FGFR2IIIc is common in mesenchymal cells, including the DTS cells (unpublished results). However, DTS cell–derived HSPG failed to support the binding of FGF7 to FGFR2IIIc whereas HSPG from DTE cells supported FGF7 binding to the FGFR2IIIc. Thus, a cell- and FGFR2IIIc-specific HSPG insures that FGFR2IIIc- and FGF7-expressing stromal cells do not exhibit an autocrine response to FGF7. A mutually exclusive splice switch results in loss of expression of FGFR2IIIb and appearance of FGFR2IIIc during progression of malignant cells from the nonmalignant DTE tumor cells (Yan et al., 1993; Feng et al., 1997). Our current results predict that the splice switching from FGFR2IIIb to FGFR2IIIc in epithelial cells may result in a gain of response to FGF2, with some effect on the response of cells to FGF7. Lastly, it is noteworthy that DTS-cell HSPG, and to some extent DTE-cell HSPG, failed to support the binding of FGF1 to the FGFR1 that is resident in the prostate stromal cells. This suggests that FGF2 may be the more significant activator of resident FGFR1 in stromal cells whereas FGF1 acts on FGFR2IIIc.

From these results, we conclude that the directional specificity of the paracrine communication from the stroma to the epithelium is determined by cell- and FGFR2 isotype–specific heparan sulfates that cooperate with the cell-specific expression of FGFR2IIIb and FGFR2IIIc–FGF7 in epithelial and stromal cells, respectively. Our results further confirm that in the absence of the mammalian cell membrane context, when heparin is artificially substituted for cellular heparan sulfate, an absolute selectivity for FGF1, FGF2, or FGF7 is encoded only in the FGFR1IIIc protein structure, but not in that of FGFR2IIIb or FGFR2IIIc. The specificity of FGFR2IIIb for FGF7 is a partnership between epithelial cell–specific FGFR2IIIb and an FGFR2IIIb-specific heparan sulfate residing in either cell type that rejects or fails to permit the binding of FGF2. The specificity of stromal cells for FGF2 is a partnership between stromal cell–specific FGFR2IIIc and a stromal cell–specific heparan sulfate that fails to support or prevents FGF7 binding. Diverse missense mutations in FGFR2 cause diverse combinations of the craniofacial and limb abnormalities associated with Apert's syndrome which appear to arise because of gain-of-function in FGFR2 signaling (Oldridge et al., 1999; Yu et al., 2000). Recently, it has been proposed from models of structures of mutant FGFR2IIIc and FGF2 complexes devoid of heparin or cellular heparan sulfate that diverse missense mutations in FGFR2IIIc cause the diverse phenotypes by causing alterations in specificity or affinity for different FGFs, independent of heparin or heparan sulfate (Yu et al., 2000; Ibrahimi et al., 2001). Our results show that both wildtype FGFR2IIIb and FGFR2IIIc are quite plastic with respect to FGF2 and FGF7 binding when out of cell context, devoid of cellular heparan sulfate. We have proposed that cellular heparan sulfate plays a dual role in the conformation of the oligomeric FGFR signaling complex. This includes maintenance of it in an inactive FGF-dependent state, in the absence of FGF, and as a requirement for binding of and determinant of specificity for activating FGF under physiological conditions (Kan et al., 1996, 1999; Uematsu et al., 2000). Mutation site–dependent graded increases in activity of the FGFR2 oligomeric signaling complex occur likely by disruption of the cell context–dependent cooperative relationships between FGFR2 and cellular heparan sulfate. This can occur by constitutive FGF-independent activity of a portion of signaling complexes or changes in FGF-specificity or affinity (McKeehan et al., 1998). This model predicts that null mutations that alter FGFR-specific heparan sulfates, with respect to quantity or quality, will also result in the gain-of-function of FGFR signaling complexes.

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