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FGF signaling, extracellular matrix and development: Genetic studies

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We study the function of fibroblast growth factor receptors (FGFRs), as tools of cellular communication. Here we analyze common basic functions of these genes and dissect in vitro roles of specific FGFR isotypes.

Molecular cell biology tends to look at cell function as cross talk from cell to cell in the organism. In fact many important interactions take place between cells and the extra cellular matrix (ECM) around them. Thus, information flows also from cell to matrix and from matrix to cell, following the highly conserved structure of our tissues. From hydra to man we are made of cell sheets separated by a mat-like ECM, the basement membrane (BM). The BM contains many molecules, including structural proteins, the laminin and collagen IV isotypes and various glycoproteins rich in heparan sulfates (HS). Laminins and type IV collagens provide the BM's mat-like meshwork. They are anchored by integrin and dystroglycan receptors, which transfer signals to the interior of the cell. The HS of the BM bind signaling molecules, such as FGFs and the EGF, PDGF, wnt and hh families. These polypeptide growth factors are important in development and cancer. Hence the BM is a platform for cellular signaling. The BM in addition is a boundary separating epithelial and mesenchymal compartments. During cell migration and cancer metastasis these boundaries are broken down and rebuilt. Much is known about the breakdown of the BM, while its positive regulation is little understood.

We encountered this problem while using a simple model to analyze the role of FGF signaling in epithelial differentiation. Embryonic stem (ES) cell-derived embryoid bodies develop when the cells are prevented from adhering to the culture dish. They are made of two cell layers separated by a BM and surround an apoptotic cavity. We introduced truncated FGFR2 cDNA into ES cells and found that this dominant negative mutation, which can inhibit multiple FGFR isotypes, inhibits differentiation. The mutant failed to form the two cell layers, had no BM and did not cavitate. We also found that the PI3K-Akt/PKB rather than the MAPK/ERK pathway was defective in this mutant (Chen et al. 2000). In mixed cultures it was revealed that the dominant negative mutant cannot produce, but can respond to an extra cellular differentiation factor made by the wild type. Seeking extracellular products to rescue the mutant, we found that highly purified laminin-1 partially rescued differentiation. It follows that FGF signaling is required for BM formation, which is necessary for embryoid body differentiation. The mutant synthesized a number of BM proteins, but failed to transcribe chains of laminin and type IV collagen isotypes (Li et al. 2001a). Hence we assumed that FGF signaling, possibly through the PI3K pathway, regulates the synthesis of laminin and collagen IV isotypes providing a framework for the BM. To test this assumption constitutively active PI3K-p110 or Akt/PKB was introduced into wild type ES cells. The result was a 50-60-fold increase in laminin and type IV collagen mRNA and protein levels. Evidence for the transcriptional regulation of these genes was obtained in co-transfection experiments with laminin beta1-luciferase, or collagen IVa1-luciferase constructs. Indeed, dominant negative Akt/PKB inhibited naturally induced laminin and collagen IV transcription in differentiating myoblasts or insulin induced insulin receptor bearing CHO-T cells (Li et al. 2001b).

These results demonstrate that signaling through PI3K and Akt/PKB is a positive control mechanism for BM formation. Because of the importance of BMs in development, angiogenesis and cancer, and the role of Akt as a strongly anti-apoptotic oncogene, we will concentrate on the analysis of this hypothesis.

FGFRs have transcriptional variants that use alternatively exon 8 or 9 to encode a part of the ligand-binding domain. This alternative exon usage defines the binding specificity and developmental localization of FGFR variants. Loss of function mutants of epithelial variant Fgfr2IIIb, have no limbs and lack the bronchial tree of the lungs (Arman et al., 1999). To investigate the cellular mechanism of the limb defect, chimeric embryos were created from LacZ labeled homozygous mutant ES cells and normal embryos. Mutant cells did not contribute to the apical ectodermal ridge (AER), the signaling center for limb outgrowth. They did not express AER markers (Fgf8, Msx1 and CD44), and interfered with expression of dorsal (Wnt-7A) and ventral (En1) ectoderm markers. It follows that the mutant inhibits migration of AER fated wild type cells along the limb bud

ectoderm. The latter finding is in accord with the requirement for FGF signaling in morphogenic cell migration and supports the notion of close association between FGF signaling and the ECM (Gorivodsky and Lonai, submitted).

To investigate the in vivo function of the Fgfr2IIIc variant, we introduced a point mutation to exon 9 creating frame shift and stop codons. Recessive viable and fertile dwarfism with skull base synostosis was obtained. Late mineralization of the entire skeleton, decrease in the expression of Spp1 (osteopontin), and Cbfa1 indicated defective osteoblast differentiation. In addition localized expression of endochondral osteogenesis markers, PTHrP and Ihh, was also inhibited. It follows that Fgfr2IIIc is a positive regulator of bone development. In this sense Fgfr2IIIb, which is expressed in the osteogenic front, differs from the inhibitory effect of Fgfr3, in the proliferating chondrocyte zone. We think that the two FGFRs cooperatively regulate bone growth and the genetically determined proportions of the skeleton. This cooperation may regulate the shape and size of the vertebrate body (Eswarakumar et al. submitted).

Selected Publications

Arman, E., Haffner-Krausz, R., Gorivodszky, M. and P. Lonai (1999) FGFR2 is required for limb outgrowth and lung branching morphogenesis. Proc. Natl. Acad. Sci. USA 96, 11895-11899.

Chen, Y., Li, X., Eswarakumar, J.P., Seger, R. and Lonai P. (2000) Fibroblast growth factor (FGF) signaling through PI 3-kinase and Akt/PKB is required for embryoid body differentiation. Oncogene 19, 3750-3756

Givol, D., Eswarakumar V. and Lonai, P. (2001) Molecular and cellular biology of FGF signaling. In: Molecular Basis of Inborn Errors of Development, C. Epstein, R. Erickson and A. Winshaw-Boris editors, Oxford University Press (in press).

Li, X., Chen, Y., Schéele, S., Arman, E., Haffner-Krausz, R., Ekblom, P. and Lonai, P. (2001) Fibroblast Growth Factor Signaling and Basement Membrane Assembly Are Connected during Epithelial Morphogenesis of the Embryoid Body. J. Cell Biol. 153, 811-822.

Li, X., Talts, U., Talts, J. F., Arman, E., Ekblom, P. and Lonai, P (2001) Akt/PKB regulates laminin and collagen IV isotypes of the basement membrane. Proc. Natl. Acad. Sci. USA (in press).

Acknowledgements

Research in our lab is supported by Israel Science Fund, ROTRF (Roche, Basel) and Prochon, Biotechnology Ltd.