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Laminin-binding integrins in rat lens morphogenesis and their regulation during fibre differentiation☆

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Abstract

Mammalian lens development involves cell–cell and cell–ECM interactions. As integrins are a major family of cell adhesion molecules, we examined the expression patterns of several integrin subunits (α3A, α3B, α6A, α6B, β1 and β4) during rat lens development. RT-PCR, in situ hybridisation, immunofluorescence and immunoblotting were used to investigate expression of integrin subunits during lens development and differentiation. RT-PCR showed expression of α3A, α6A, α6B and β1A but not α3B or β4 subunits in postnatal rat lenses. Each subunit displayed distinct spatio-temporal expression patterns. β1 integrin was expressed in both epithelium and fibres. α3A subunit expression was restricted to the epithelium; expression ceased abruptly at the lens equator. Expression of the α6A subunit increased during fibre differentiation, whereas α6B expression was predominantly associated with epithelial cells during lens development. In lens epithelial explants, FGF induced some of the changes in integrin expression that are characteristic of fibre differentiation in vivo. One notable exception was the inability of FGF to reproduce the distinctive down-regulation of the α3 isoform that is associated with initiation of elongation in vivo. Interestingly, vitreous treatment was able to reproduce this shift in α3 expression indicating that another factor(s), in addition to FGF, may be required for full and complete transition from an epithelial cell to a fibre cell. Integrin subunit expression therefore appears to be highly regulated during lens development and fibre differentiation with evidence of major changes in α3 and α6 isoform expression. These results indicate that integrins may play important roles in development and growth of the lens. How specific integrin subunits influence the behaviour of cells in different developmental compartments of the lens remains to be determined.

Keywords: integrin; cell adhesion; lens development; lens differentiation; fibroblast growth factor

1. Introduction

Lens function is dependent on a precisely ordered cellular architecture and patterns of cell behaviour that are established during embryonic development. The dramatic changes in cell–cell and cell–extracellular matrix (ECM) interactions as the lens develops and grows, implicate a role for cell adhesion molecules in these processes. The integrins represent a large family of cell adhesion molecules and their ability to transmit signals as well as mediate cell adhesion makes them prime candidates for having a role in lens development.

The embryonic lens develops from head ectoderm that associates closely with the optic vesicles. Ectodermal cells elongate in this region to form the lens placode, which subsequently invaginates to form the lens vesicle. Cells in the posterior part of the lens vesicle undergo extensive elongation to form the primary lens fibres while cells in the anterior region remain cuboidal forming the anterior epithelial monolayer (McAvoy, 1980; Piatigorsky, 1981). As the lens develops postnatally, proliferation becomes

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localized to a band of epithelial cells above the lens equator, the germinative zone (Hanna and O'Brien, 1961). Progeny of these divisions migrate or are displaced below the equator, into the transitional zone, where they elongate and differentiate into secondary lens fibres The mature lens is therefore composed of a monolayer of epithelial cells lining the anterior surface and a fibre mass posteriorly. There is compelling evidence that several growth factor families, particularly members of the fibroblast growth factor (FGF) family play key roles in regulating the patterns of proliferation and differentiation in the lens leading to its highly polarized structure (Chamberlain and McAvoy, 1997; McAvoy et al., 1999; Lang and McAvoy, 2004).

The lens is enclosed by a thick basement membrane, the lens capsule. Although the composition of this complex ECM has not yet been completely determined, major components in mammals include laminin, collagen IV, heparan sulfate proteoglycans (HSPGs), entactin/nidogen and fibronectin (Harding and Dilley, 1976; Parmigiani and McAvoy, 1984; Cammarata et al., 1986; Sawhney, 2002). Changes in distribution of some of these ECM components have been identified during lens development. For example, in rats during embryonic development, immunoreactivity for laminin is predominantly localized to the posterior region of the capsule (E10–13). However, as development progresses laminin reactivity becomes localized throughout the capsule (Parmigiani and McAvoy, 1984). Consistent with a key role for laminin in lens cell biology, embryonic and postnatal rat lens epithelial cells have been shown to migrate on a laminin substratum in vitro and retain their ability to differentiate into fibre cells when stimulated by FGF (Parmigiani and McAvoy, 1991; Hales et al., 1992).

Integrins are heterodimeric transmembrane proteins that function in cell adhesion (cell–cell and cell–ECM) and bidirectional signalling. Each heterodimer consists of one α and one β subunit that associate non-covalently through their extracellular domains to produce an active protein. Both the cytoplasmic and extracellular domains of several integrin subunits can be alternatively spliced, giving rise to a number of different isoforms. The expression of individual isoforms often displays spatio-temporal regulation during the growth and development of various tissues (Jiang and Grabel, 1995; Thorsteinsdottir et al., 1995; Zhidkova et al., 1995; de Melker et al., 1997).

The importance of integrins during early chick ocular morphogenesis has been demonstrated by injection of RGD-containing peptides or β1 antibodies that block integrin function, disrupting the invagination of the optic cup and lens vesicle (Svennevik and Linser, 1993). Integrins have also been shown to be expressed in distinct spatio-temporal patterns in the chick lens and implicated in the fibre differentiation process (Menko and Philip, 1995; Menko et al., 1998; Walker and Menko, 1999). However, little information is available on the repertoire of integrins that are expressed during mammalian lens development. Based on the composition of the lens capsule, the ability of lens cells to migrate and differentiate on laminin, and previous studies on chick lens, we investigated the expression of laminin-binding integrin subunits (α3, α6, β1 and β4) during rat lens development. We also used lens explant cultures to study regulation of integrin expression during fibre differentiation.

2. Methods

2.1. Animal tissues

Albino Wistar rat embryos were collected on days 12–18 of gestation (E12–E18) after timed matings of female rats in pro-oestrous. Embryos and eyes from postnatal day 3 (P3) and 21 (P21) rats were fixed in 10% neutral buffered formalin (NBF) and embedded in paraffin. E14 rat embryos, P21 rat skin and whole lenses from newborn (P2) and weanling (P21) rats were also collected, frozen and stored at −80°C for subsequent RNA extraction.

2.2. Tissue culture

Lens epithelial explants were prepared from 10-day-old rats as described (Liu et al., 1994) and cultured in medium 199 with or without recombinant human FGF-2 (100 ng/ml; PeproTech, Rocky Hill, NJ) for 8 days at 37°C. Medium with or without FGF was replaced after 5 days culture. At the end of the culture period, explants were pooled according to treatment, and stored at −80°C until RNA extraction for RT-PCR analysis.

Additional explants were also set up from 10-day-old rats and cultured for 8 days and 25 days (FGF) or 35 days (vitreous) with either 100 ng/ml FGF-2 or bovine vitreous diluted 1:2 with medium 199. At the end of the culture period, explants were fixed for 10 min in 10% NBF, washed in PBS and embedded in paraffin.

2.3. RT-PCR

Total RNA was isolated from whole E14 rat embryos, P2 and P21 rat lenses and from pooled lens epithelial explants using TRIzol reagent (Life Technologies, Sydney, Australia). First strand cDNA synthesis was carried out on 1–2.5 μg of RNA using a reverse transcription system (Promega, Sydney, Australia) according to the manufacturer's instructions. In control reactions AMV reverse transcriptase was omitted. Primer pairs were designed to span exon–intron boundaries, amplifying only cDNA. Aliquots (2 μl) of each reverse transcription mix were amplified for 28–35 cycles (94°C for 30 sec, 60°C for 30 sec, 72°C for 1 min), followed by a final extension at 72°C for 2 min, using specific primers for α3, α6, β1, β1A-D, β4A-C and β4E integrin subunits and GAPDH (Table 1).

Primers for α3 and α6 detect and distinguish between the A and B isoforms for each respective subunit. The primers for β1 amplify a region in the transmembrane or