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Desmosomal adhesiveness is developmentally regulated in the mouse embryo and modulated during trophectoderm migration

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ABSTRACT

During embryonic development tissues remain malleable to participate in morphogenetic movements but on completion of morphogenesis they must acquire the toughness essential for independent adult life. Desmosomes are cell–cell junctions that maintain tissue integrity especially where resistance to mechanical stress is required. Desmosomes in adult tissues are termed hyper-adhesive because they adhere strongly and are experimentally resistant to extracellular calcium chelation. Wounding results in weakening of desmosomal adhesion to a calcium-dependent state, presumably to facilitate cell migration and wound closure. Since desmosomes appear early in mouse tissue development we hypothesised that initial weak adhesion would be followed by acquisition of hyper-adhesion, the opposite of what happens on wounding. We show that epidermal desmosomes are calcium-dependent until embryonic day 12 (E12) and become hyper-adhesive by E14. Similarly, trophectodermal desmosomes change from calcium-dependence to hyper-adhesiveness as blastocyst development proceeds from E3 to E4.5. In both, development of hyper-adhesion is accompanied by the appearance of a midline between the plasma membranes supporting previous evidence that hyper-adhesiveness depends on the organised arrangement of desmosomal cadherins. By contrast, adherens junctions remain calcium-dependent throughout but tight junctions become calcium-independent as desmosomes mature. Using protein kinase C (PKC) activation and PKCα−/− mice, we provide evidence suggesting that conventional PKC isoforms are involved in developmental progression to hyper-adhesiveness. We demonstrate that modulation of desmosomal adhesion by PKC can regulate migration of trophectoderm. It appears that tissue stabilisation is one of several roles played by desmosomes in animal development.

Introduction

An important contrast between embryonic and adult tissues is that the former must be labile to permit modelling by morphogenetic movement whereas the latter must be tough to resist the rigours of independent existence. The change from lability to toughness is therefore an important aspect of the developmental process. This paper examines how cell-cell adhesion changes during mouse development to increase tissue strength.

Desmosomes are intercellular adhesive junctions necessary for the stability and integrity of tissues (Dusek et al., 2007; Garrod and Chidgey, 2008; Garrod and Kimura, 2008; Holthofer et al., 2007; Kottke et al., 2006; Schmidt and Koch, 2007; Thomason et al., 2012; Waschke, 2008). In cultured cell sheets, desmosomes progress from a weakly adhesive, calcium-dependent form to a more strongly adhesive, calcium-independent form, termed hyper-adhesive (Garrod et al., 2005; Kimura et al., 2007; Mattey and Garrod, 1986; Wallis et al., 2000; Watt et al., 1984). This progression occurs without any detectable change in the molecular composition of desmosomes (Kimura et al., 2007). Hyper-adhesion is associated with the presence of an electron-dense midline in the intercellular space between the plasma membranes that represents a highly ordered arrangement of the desmosomal cadherins (Al-Amoudi et al., 2007; Garrod et al., 2005).

In the mouse embryo, desmosomes form first in the cavitating blastocyst and subsequently appear early in the development of various tissues including epidermis and kidney tubule (Davies and Garrod, 1995; Fleming et al., 1991; Gallicano et al., 1998;

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Garrod and Fleming, 1990). In adults, desmosomes are present in all epithelia and cardiac muscle, the majority or all of them being hyper-adhesive (Garrod et al., 2005; Wallis et al., 2000). As such they act as strong intercellular links in the desmosome-intermediate filaments complex, malfunction of which, in disease and experimental animals, causes tissues to disintegrate (Fuchs, 1992; Garrod and Chidgey, 2008; Jonkman et al., 2005; Lane and McLean, 2004; Stanley and Amagai, 2006; Vasioukhin et al., 2001).

In adult tissues, controlled down-regulation of intercellular adhesiveness is sometimes required. For example, in skin wound healing, keratinocytes must migrate to close the wound and remodel the epidermis (Krawczyk and Wilgram, 1973). In wound edge epidermis, as in wounded cell sheets in culture, hyper-adhesive desmosomes revert to calcium-dependence (Garrod et al., 2005; Wallis et al., 2000). This reversion involves activation of the conventional protein kinase C isozyme PKCα, which localises to desmosomal plaques in wound edge epidermis (Garrod et al., 2005; Wallis et al., 2000).

Desmosomal adhesion is essential for mouse development from the early pre-implantation stage (Gallicano et al., 1998). Because of the requirement for tissue lability during development it seemed unlikely that such early desmosomes would be hyper-adhesive. We show that acquisition of hyper-adhesion is developmentally regulated in epidermis. Curiously, hyper-adhesion also develops in the trophectoderm of the late blastocyst. We present evidence suggesting that these events may be regulated by conventional PKC isozymes and that modulation of desmosomal adhesiveness by PKC can regulate trophectoderm migration.

Materials and methods

Mouse strains and preparation of post-implantation embryos

Embryos were obtained from 6–16 week old MF1 female mice (Charles River Ltd, Margate, UK). The time of discovery of the vaginal plug was regarded as embryonic day (E) 0. At the appropriate time, mice were killed by neck dislocation, the abdomen opened, and embryos were carefully dissected into PBS. The back skin was dissected from E14 and older embryos. PKCα−/− mice were kindly gifted by Dr Peter Parker (Leitges et al., 2002). Experiments were carried out in the same way as MF1 mice.

Mouse strain, blastocyst collection and culture

Blastocysts were obtained from 8–12 week old MF1 mice after superovulation by intraperitoneal injection of 5 i.u. of pregnant mare serum gonadotrophin (PMS; Sigma, Missouri, USA) followed by 5 i.u. human chorionic gonadotrophin (hCG; Calbiochem) 48 h later and mating. The time of discovery of the vaginal plug was regarded as embryonic day (E)0. E3.5 early blastocysts (cavity $<20\%$ volume) were flushed from dissected oviducts with M2 medium (GIBCO, Paisley, UK) supplemented with 4 mg/ml BSA (M2-BSA) and cultured in M6 medium (GIBCO, Paisley, UK) containing 4 mg/ml BSA up to the middle blastocyst stage ($20\% <$ cavity $< 50\%$ volume, E4) and late blastocyst stage (cavity $> 50\%$ volume, E4.5). Culture was performed in 5% CO_2 at 37 °C in microdrops under mineral oil (Sigma, Missouri, USA) as previously described (Sheth et al., 1997).

Low calcium medium (LCM) treatment

Entire embryos (until E13.5) or isolated back skin pieces (1 cm × 1 cm) (from E14) were incubated in Dulbecco's Modified Eagle's Medium (DMEM) plus 10% foetal bovine serum (FBS) (Standard medium=SM) or calcium-free DMEM containing 3 mM EGTA plus 10% chelated FBS (Low calcium medium=LCM) for 2 h (E10) or 4 h in 5% CO_2 at 37 °C.

The zonae pellucidae were removed from blastocysts by incubating for 15–30 s in acid Tyrode's medium (Sigma) at 37 °C before washing in M2-BSA. (Sigma, Missouri, USA). Zona-free blastocysts were incubated in LCM or SM as control in 5% CO_2 at 37 °C for 1 h (early and middle blastocysts) or 2 h (late blastocysts). The trophectoderm of late blastocysts was pierced once or twice with a fine glass needle during incubation to allow blastocoel cavity collapse and medium access. After treatment, blastocysts were fixed in 1% PFA (paraformaldehyde, Sigma, Missouri, USA) in PBS for 10 min at room temperature and used for immunostaining.

PKC inhibitor treatment

Samples from E12 embryos were incubated with 10 or 50 nM Gö6976 (Sigma, Missouri, USA) or PKCβ inhibitors, 50, 100 or 200 nM 3-(1-(3-imidazol-1-ylpropyl)-1H-indol-3yl)-4-anilino-1H-pyrrole-2,5-dione or 10 or 20 nM LY 333531 (Calbiochem, Beeston, UK) for 1 h. Controls were incubated with DMSO (1/1000 in culture medium). Samples were then washed three times with PBS and subjected to LCM treatment.

After recovery from zona pellucida removal in M2-BSA for 10 min, early blastocysts were incubated with 50 nM or 100 nM Gö6976 (Sigma, Missouri, USA) in M2-BSA at 37 °C for 1 h. Controls were incubated with DMSO (1/100).

Cryosectioning and fixation

Frozen embryos or isolated skin embedded in OCT were cryosectioned at 10 μm using a Lieca Reichert-Jung Cryocut 1800 cryostat and placed onto microscope slides. Samples were fixed by incubation in ice-cold acetone/methanol (1:1) for 20 min at −20 °C.

Immunostaining

The antibodies used in this study are listed in Table 1. Sections were washed in PBS 3 × 5 min. A wax pen (DACO) was used to divide sections on a slide. Non-specific staining was blocked with 10% (v/v) goat serum, in PBS for 30 min, and then incubated on 20 μl droplets of primary antibody diluted in PBS for 1 h. Samples were washed 3 × 5 min in 200 μl droplets of PBS, then incubated with 20 μl of secondary antibodies diluted in PBS for 1 h. Nuclei were stained by incubating with 1/1000 diluted Hoechst stock (5 mg/ml, Sigma, Missouri, USA) together with the secondary antibodies. After washing as before, samples were mounted on microscope slides using Gelvatol (Fluka).

Fixed blastocysts were placed on coverslips coated with poly-L-lysine hydrobromide (Sigma, Missouri, USA) and attached onto stainless steel washers (internal diameter 7 mm, external diameter 20 mm, thickness 1.25 mm, Woodside, Manchester, UK). They were processed for immunocytochemistry as previously described (Sheth et al., 1997). After fixation, blastocysts were

Table 1
Antibodies used in this study.

Antigen	Antibody	Source or reference
Desmoplakin	11–5F, monoclonal	(Parrish et al., 1987)
E-cadherin	monoclonal	U3254, Sigma
β-catenin	polyclonal	C-2208, Sigma
ZO-1	polyclonal	617300, Zymed Laboratories Inc.