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Centrosome Reorientation in Regenerating Endothelial Monolayers Requires bFGF

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Abstract Monolayers of endothelial cells respond to physical denudation with a characteristic sequence of lamellipodia extrusion, cell migration, and cell proliferation Basic fibroblast growth factor (bFGF) has been implicated as a necessary component of this process addition of exogenous bFGF enhances monolayer regeneration both in vitro and in vivo, and monolayer regeneration can be inhibited in vitro by treatment with neutralizing antibodies raised against bFGF Centrosome reorientation from a random location to one preferentially situated between the nucleus and the denudation edge has been postulated as a mechanism essential for cell polarization and subsequent migration This present study examined the effects of a polyclonal antibody to bFGF and suramin on monolayer regeneration, actin microfilament staining, and centrosome orientation at the wound edge of partially denuded bovine large vessel endothelial monolayers. Treatment with anti-bFGF or suramin abolished monolayer repair in these cultures Cells at the denudation edge showed altered actin staining patterns and reduced lamellipodia extrusion, and there was complete inhibition of centrosome reorientation in treated cultures Monolayer repair and centrosome reorientation could be restored by addition of exogenous bFGF in antibody but not suramin treated cultures Recent evidence suggests that preferential centrosome location in migrating cells may be a consequence of lamellipodia protrusion and cell spreading, rather than an indication of cell polarization However, these results indicate that agents which interfere with bFGF availability prevent endothelial monolayer regeneration via mechanisms involving cell spreading and/or centrosome reorientation 1993 Wiley Liss Inc

Key words: endothelium, wound repair, basic fibroblast growth factor, suramin

Basic fibroblast growth factor (bFGF) is a polypeptide produced as an autocrine or paracrine factor by a number of different cell types including endothelial cells. Although this molecule lacks a classic signal sequence on its amino terminal, it is nonetheless exocytosed from cells by mechanisms as yet not fully characterized [Mignatti et al., 1992]. Extracellular matrix components, particularly heparan sulfate proteoglycans, act as a storage depot or sink for exocytosed bFGF [Flaumenhaft et al., 1989; Presta et al., 1989; Ruoslahti and Yamaguchi, 1991]. Studies with vascular endothelial cells suggest that bFGF may be released via membrane disruptions during cell migration or due to microwounding of cells in culture [McNeil et al., 1989; Muthukrishnan et al., 1991].

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Many types of vascular endothelium require exogenous bFGF or bFGF-like molecules to survive in culture, although others may produce and release sufficient amounts endogenously. Endothelial cell migration, either directed as individual cells moving towards a chemotactic stimulus or "induced" as by the loss of contact inhibition in a confluent monolayer, also requires bFGF [Tsuboi et al., 1990]. Numerous studies have examined endothelial monolayer repair using cultured vascular endothelial cells induced to proliferate by physically denuding a portion of the culture. The subsequent repair requires cell spreading and cell migration in addition to proliferation [Coomber and Gotlieb, 1990; Gotlieb, 1990; Muthukrishnan et al., 1991].

Addition of exogenous bFGF will accelerate the repair process, and monolayer regeneration may be reduced or eliminated by treating cultures with neutralizing antibodies to bFGF [Sato and Rifkin, 1988; Tsuboi et al., 1990]. The centrosome consisting of microtubule organizing

centre (MTOC) and associated structures reorients from a previously random location to one between the nucleus and forward edge of the spreading cell during epithelial monolayer regeneration. This has been proposed as an early event involved in polarization of cells for subsequent migration [Singer and Kupfer, 1986; Gotlieb, 1990]. Recent evidence suggests that this phenomenon is more a reflection and consequence of cell spreading and lamellipodia protrusion, rather than an active process [Euteneuer and Schliwa, 1992]. Thus centrosome location in cells at the edge of a denuded monolayer of endothelium is a valid indication of lamellipodia extrusion and cell spreading, events essential for successful monolayer regeneration.

A previous study examined the effects of exogenous bFGF on bovine pulmonary artery endothelial cell (BPAEC) [Coomber, 1991]. These cells are able to regenerate a partially denuded monolayer in the absence of exogenous bFGF. These cells are, however, responsive to exogenous bFGF, as treatment with 10 ng/ml results in enhanced monolayer regeneration. No detectable enhancement of either cell proliferation or centrosome relocation was seen in cells at the denudation edge, suggesting that in contrast to many types of vascular endothelium these BPAEC may produce/release sufficient bFGF for normal regeneration of denuded monolayers. An alternative is that these cells may not require bFGF for monolayer regeneration. This hypothesis is examined in the present study, which quantifies aspects of BPAEC regeneration in the presence of neutralizing antibodies to bFGF.

METHODS

BPAEC, CCL 209 from American Type Culture Collection, were plated onto sterile glass coverslips and cultured in Eagle's minimal essential medium (MEM) and 10% FBS until confluent. Cultures were partially denuded and monolayer regeneration was monitored as previously described [Coomber and Gotlieb, 1990; Coomber, 1991]. MTOC was localized at the denudation edge in monolayers stained for tubulin via indirect immunofluorescence after various incubation times. Paraformaldehyde fixed cultures were incubated with mouse monoclonal anti-tubulin [Leslie et al., 1984] followed by fluorescein conjugated goat anti-mouse IgG (Sigma, St. Louis, MO). MTOC localization was scored relative to the nucleus and leading edge of cells and the denudation border. Cells were considered to have MTOC oriented towards the wound if the MTOC was located within the front half of the cell. Golgi was localized in a similar manner in regenerating cultures by incubating with 2.5 μg/ml C_5-DMB-ceramide [Pagano et al., 1989] (Molecular Probes, Inc., Eugene, OR) in 0.4 mg/ml fatty acid free BSA at 37°C for 5 min, followed by several rinses in BSA containing media and subsequent paraformaldehyde fixation. For both tubulin and ceramide stained monolayers at least 100 cells were scored for each wound edge and results were expressed as percent of total.

G-actin was visualized in wound edge cells of paraformaldehyde fixed monolayers after staining with DNase I (Molecular Probes, Inc.). Triton permeabilized cells were incubated with 0.3 μM fluorescein conjugated DNase I for 20 min, rinsed, and mounted. Additional regenerating monolayers were stained for F-actin with 5 ng/ml rhodamine conjugated phalloidin (Sigma) using similar fixation and incubation techniques.

Control cultures were incubated with MEM containing 0.5% FBS plus 5 μg/ml non-specific rabbit IgG (Sigma, reagent grade). Experimental monolayers were incubated with MEM containing 0.5% FBS ± 5 μg/ml polyclonal anti-bFGF (R & D Systems) or ± 0.1 mM suramin (Miles, West Haven, CT) for up to 24 h after denudation. Suramin is a polyanionic compound which interferes with growth factor/receptor interactions and cell signaling. It has been shown to interact with bFGF, as well as other polypeptide growth factors [Cardinali et al., 1992; Eisenberger and Fontana, 1992; Minniti et al., 1992]. Additional cultures were treated with the above concentrations of antibody or suramin in the presence of 10 ng/ml bovine recombinant bFGF (Boehringer Mannheim, Montreal).

All experiments were performed at least in triplicate, and values were pooled to calculate mean and standard error of mean. ANOVA was used to examine differences within and among groups, and Student's t-test ($\alpha = 0.05$) was used to compare means where F values were significant at the 5% level.

RESULTS

Treatment of partially denuded BPAEC monolayers with antibodies to bFGF or with 0.1 mM suramin abolished wound repair and prevented centrosome orientation to the leading edge of the cell, suggesting that these cells require en-

dogenous bFGF for their regenerative response in vitro.

Monolayer integrity was minimally affected by the doses of suramin and anti-bFGF used in these studies, although there was some loss of cells after several hours incubation with suramin (Fig. 1). Preliminary studies using 0.25 mM suramin with low serum conditions resulted in considerable disruption in monolayer integrity (data not shown). Lamellipodia extrusion and cell spreading as revealed by phase contrast microscopy was reduced in treated cultures (Fig. 1). Lamellipodia extrusion is an active process in locomoting cells which involves actin microfilaments. It is possible that less lamellipodia extrusion occurs in treated cultures in this study due to insufficient actin polymerization at the lamellipodia edge. Staining of regenerating monolayers with DNase I suggests that G-actin levels may be reduced at lamellipodia edges in anti-bFGF and suramin treated cultures (Fig. 2). Filamentous actin staining patterns were also altered in treated cultures. Stress fibers were numerous and oriented perpendicular to the denuded edge in control cells, but cells from anti-bFGF and suramin treated cultures showed remnants of the dense peripheral band of actin seen in confluent monolayers [Coomber and Gotlieb, 1990] and stress fibers oriented parallel to the denudation edge (Fig. 2).

Monolayer regeneration was significantly reduced ($P < 0.05$) in cultures treated with neutralizing antibodies or with suramin (Fig. 3). Control cultures showed a linear response, with increasing monolayer regeneration with incubation time. In treated cultures, there was some initial retraction of the monolayer but by 8 h incubation there was limited cell spreading, and subsequently some monolayer repair. No significant differences ($P < 0.05$) in monolayer repair were seen between anti-bFGF and suramin treated cultures. Monolayer repair could be restored to control levels by incubating antibody treated cultures simultaneously with 10 ng/ml bFGF (Fig. 3A). Monolayer repair could not be restored in suramin cultures by similar treatment with exogenous bFGF (Fig. 3B).

Centrosome reorientation occurs in these BPAEC even under the low serum conditions of these studies. These experiments examined the location of golgi and MTOC relative to the leading edge of cells in cultures incubated for up to 12 h after denudation (Fig. 4). MTOC and golgi localization towards the leading edge of the cell is rapid and sustained in control cultures (Fig. 5). Treatment with either anti-bFGF or suramin consistently and significantly abolishes this response ($P < 0.05$). As with monolayer repair,

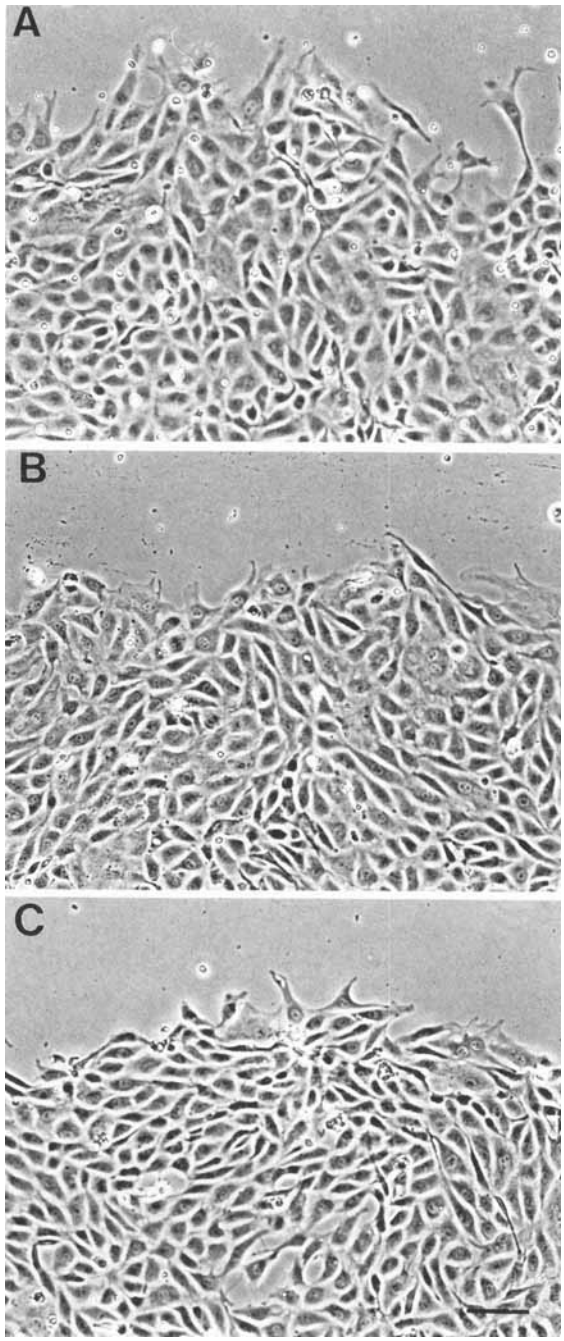

Fig. 1. Phase contrast micrographs of regenerating monolayers 8 h after denudation Direction of monolayer repair is towards the top Cells in control cultures (**A**) are elongated with long lamellipodia protruding into the denuded region Cells in cultures incubated with 5 μg/ml anti-bFGF (**B**) or with 0 1 mM suramin (**C**) are rarely elongated in the direction of monolayer regeneration and are poorly spread Scale bar = 100 μm

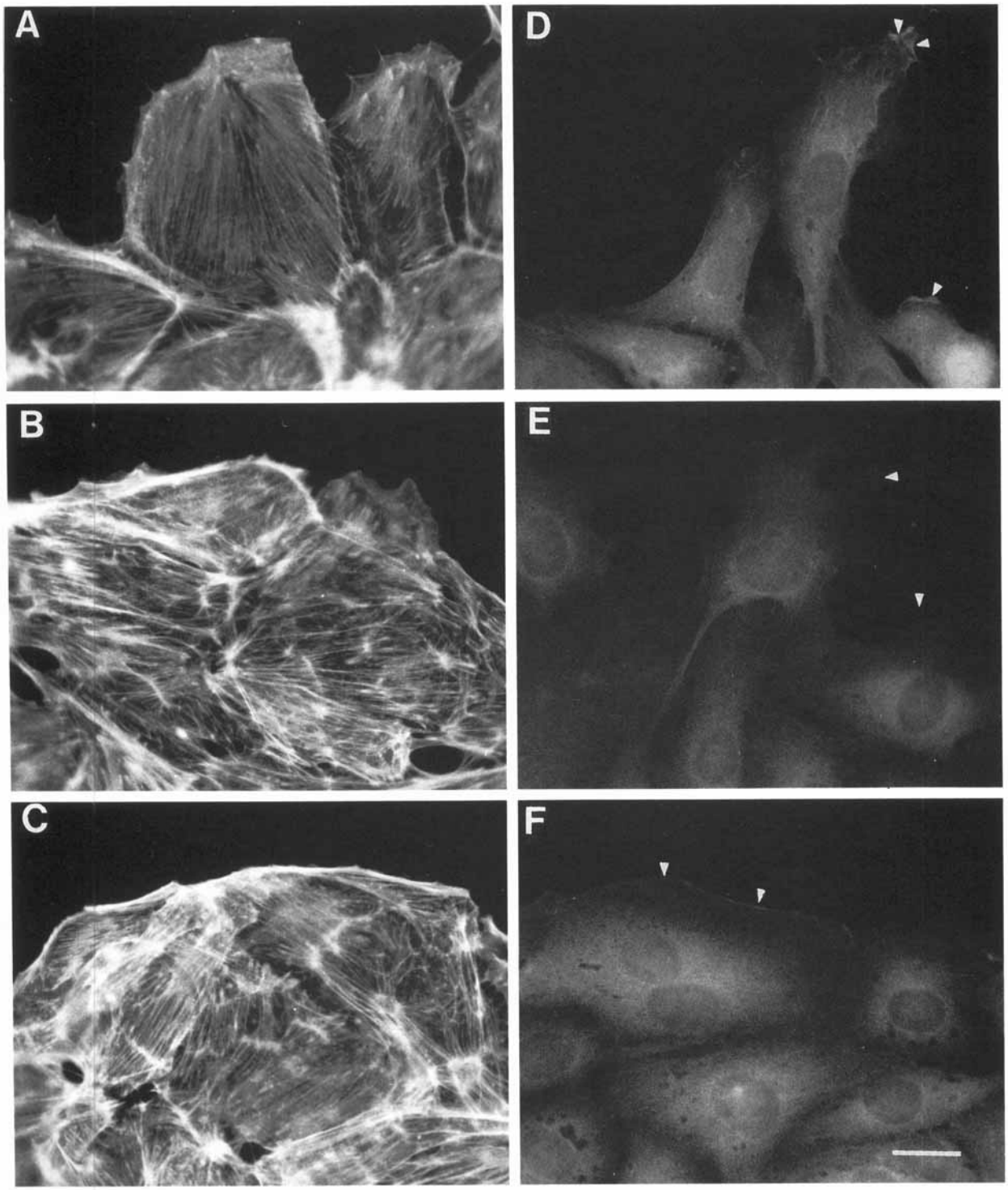

Fig. 2. Regenerating endothelial monolayer after 8 h incubation, stained for filamentous actin (**A–C**) or globular actin (**D,F**) Control cultures (A,D) have numerous actin stress fibers oriented parallel to lamellipodia extrusion, and G-actin accumulations at their lamellipodia edges (arrowheads) Treatment with anti bFGF (B,**E**) or suramin (C,F) reduces lamellipodia spreading Such cells have fewer and less organized actin stress fibers and reduced amounts of G actin located at their lamellipodia edges (arrowheads) Scale bar = 10 μm

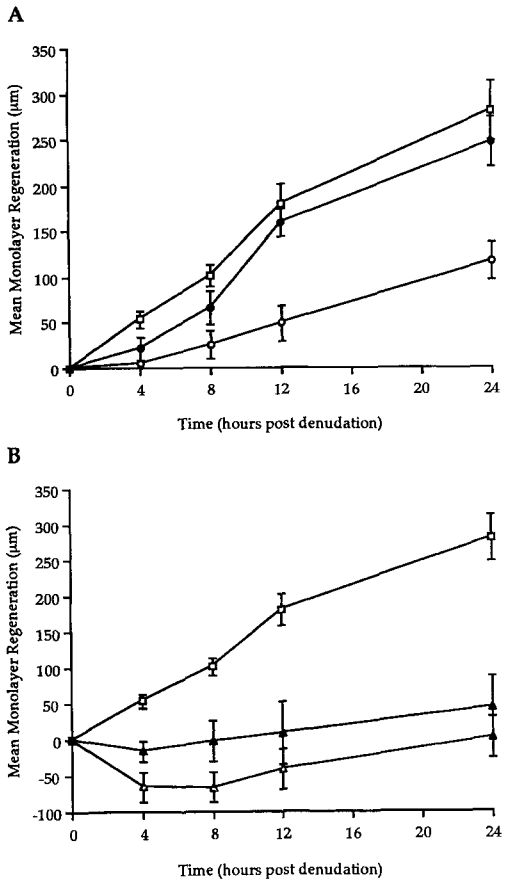

Fig. 3. Quantification of monolayer regeneration in control and treated cultures. Control (□), 5 μg/ml anti-bFGF (○), 0.1 mM suramin (△), antibody plus 10 ng/ml bFGF (●), suramin plus 10 ng/ml bFGF (▲). In both anti-bFGF (**A**) and suramin (**B**) treated monolayers, monolayer repair is significantly reduced from control values ($P < 0.05$). Monolayer repair is restored by co-incubation of anti-bFGF treated cultures with 10 ng/ml bFGF (A), but co-incubation of suramin treated cultures with 10 ng/ml bFGF has no significant effect (B).

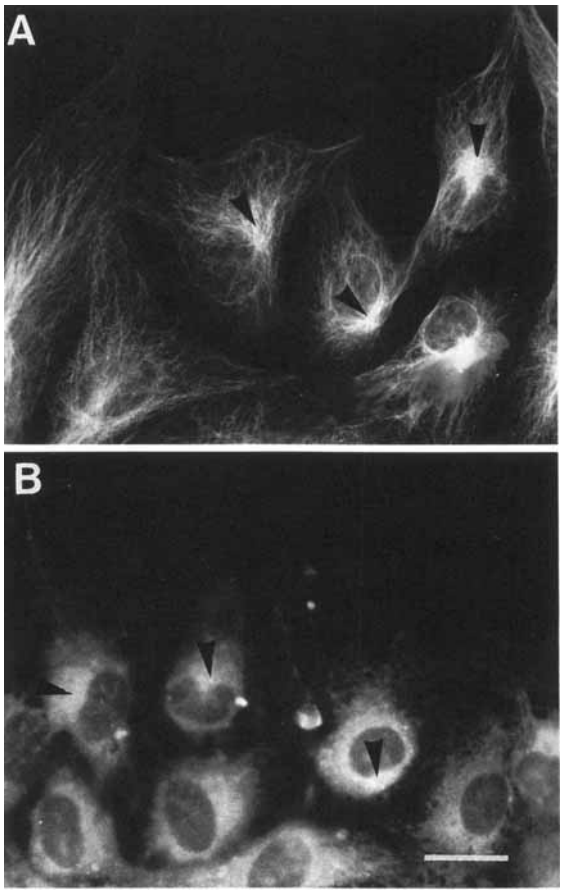

Fig. 4. Endothelial monolayers 8 h after partial denudation. Cells in **A** are stained for microtubules via indirect immunofluorescence. In **B**, a different culture is vitally stained with C_5-NBD-ceramide immediately prior to fixation. In both cases the wound edge is towards the top. Arrowheads indicate the location of MTOC (A) and golgi (B) relative to denudation edge and cell nucleus. Scale bar = 10 μm.

centrosome reorientation is restored by addition of exogenous bFGF in antibody but not in suramin treated cultures (Fig. 5).

Therefore, BPAEC exhibit monolayer regeneration in vitro under low serum conditions and in the absence of exogenous bFGF. Neutralizing antibodies to bFGF and the polyanionic drug suramin which interacts with bFGF receptors inhibit aspects of this regenerative response, specifically centrosome orientation. Responses due to neutralizing antibody are ameliorated by addition of exogenous bFGF. These data strongly suggest that BPAEC require access to endogenously produced bFGF for normal centrosome orientation and cell spreading at the wound edge and subsequent monolayer regeneration.

DISCUSSION

These studies demonstrate that treatment of regenerating endothelial monolayers with neutralizing antibodies to bFGF or with suramin eliminates centrosome orientation in wound edge cells and reduces monolayer repair. These responses can be restored in antibody treated cells by addition of exogenous bFGF.

Neutralizing antibodies to bFGF will prevent endothelial cell proliferation, as will treatment with suramin [Sato and Rifkin, 1988]. Basic FGF has been localized to the nucleus and cytoplasm of numerous cells types in vitro, and nuclear localization has been reported to occur during the transition from a quiescent to proliferating phenotype [Baldin et al., 1990; Bugler et al., 1991; Cattini et al., 1991; Dell'Era et al.,

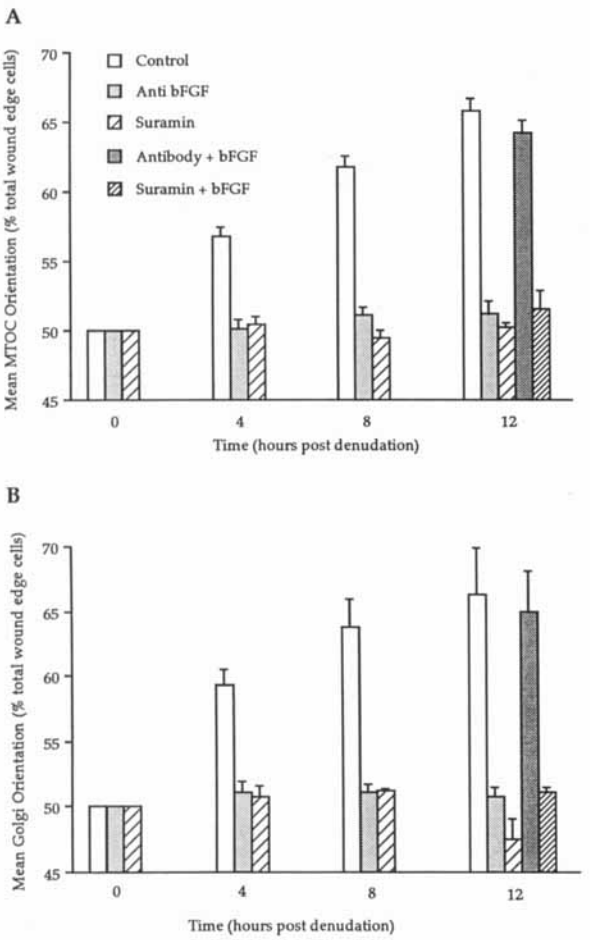

Fig. 5. **A:** MTOC location was scored relative to cell front and nucleus for cells at the denudation edge in control and treated cultures incubated for various times post wounding. In control cultures, significantly greater proportions of cells have MTOC preferentially localized to the putative front with increasing incubation times. Treated cultures show no apparent alterations in MTOC localization, and proportions of cells with MTOC localized to the front were not significantly different between anti-bFGF and suramin treated cultures ($P < 0.05$). **B:** Golgi localization was quantified in wound edge cells as described in A. There was a significant reduction in proportions of cells with golgi localized to the front relative to control in cultures treated either with anti-bFGF or with suramin ($P < 0.05$). Again, golgi localization was restored to control in antibody treated cells values by co-incubation with 10 ng/ml bFGF.

1991; Powell and Klagsbrun, 1991]. This cytokine is also essential for cell migration. Endothelial motility has been correlated with bFGF content [Tsuboi and Rifkin, 1990]. Substances such as suramin and platelet factor 4 which interfere with bFGF binding to cell surface receptors, or neutralizing antibodies to bFGF, suppress both migration of single cells [Mignatti et al., 1991] and endothelial monolayer regeneration [Sato and Rifkin, 1988; Sato et al., 1990].

Centrosome orientation towards the front of a motile cell has been suggested as a necessary component for directed cell migration [Singer and Kupfer, 1986; Gotlieb, 1990]. Previous studies with this cell line found no alterations in centrosome localization in regenerating monolayers treated with 10% FBS and exogenous bFGF [Coomber, 1991]. Recent evidence suggests that localization of the centrosome from a previously random position to one preferentially situated between the nucleus and the advancing edge of a motile cell is more a consequence of lamellipodia protrusion and cell spreading, rather than a feature of cell polarization [Euteneuer and Schliwa, 1992]. This present study therefore examined other cytoskeletal events which reflect lamellipodia extrusion, particularly the arrangement of filamentous and globular actin in the lamellipodia of denudation edge cells. Polymerization of actin filaments at the leading edge of cells has been suggested as a major mechanism for lamellipodia extrusion. Recent studies of tumor cell lines show correlation between membrane ruffling, cell spreading and lamellipodia formation, and cell motility, and the presence of actin binding protein and normal actin stress fibers [Cunningham et al., 1992]. Photoactivation studies of fibroblasts injected with fluorescent actin suggest that lamellipodia extrusion is driven by actin polymerization at the cell edge [Theriot and Mitchison, 1992].

The work presented here found qualitative differences in actin staining patterns. In particular, orientation of actin stress fibres in treated cultures is parallel to the denudation edge rather than oriented in the direction of cell spreading as is seen in control cultures, and staining for G-actin revealed a reduced amount of monomer located at lamellipodia edges in treated cultures. These results are consistent with reduced lamellipodia formation and consequent cell spreading previously reported in this regenerating monolayer model [Coomber and Gotlieb, 1990; Coomber, 1991] and suggest that inhibition of monolayer repair seen in treated cultures is at least partly due to reduced cell spreading. Decreases in cell proliferation are unlikely to account for these reductions in monolayer regeneration, as endothelial cell division is not a contributing factor in monolayer repair until at least 16 h after denudation [Schwartz et al., 1979].

Suramin is a polyanionic molecule which interacts with numerous growth factors and/or their receptors including EGF [Cardinali et al., 1992;

Lopez Lopez et al., 1992], TGF-β [Wade et al., 1992], and bFGF [Lopez Lopez et al., 1992; Pesenti et al., 1992]. In the present study, suramin effectively and persistently prevented cell spreading, monolayer regeneration, and centrosome orientation even in the presence of excessive exogenous bFGF. Studies in vivo with suramin indicate that it will bind very avidly to bFGF in the absence of high levels of serum proteins, and that this binding cannot be prevented with additional exogenous bFGF [Lopez Lopez et al., 1992]. Thus the inability of exogenous bFGF to ameliorate the effects of suramin on cells in this present study is likely due to similar mechanisms. It is also possible that interaction of suramin with growth factors/receptors other than bFGF may play a role in monolayer repair alterations.

These experiments suggest that endothelial monolayer regeneration in vitro is inhibited by interference of bFGF/receptor binding. This disruption in bFGF induced cell signaling manifests itself as reduced cell spreading and inhibition of centrosome relocalization in denudation edge cells. It is not clear if the absence of centrosome orientation seen in this study is a consequence of or a cause of reduced cell spreading, however, previous studies suggest that centrosome orientation may occur independent of obvious cell spreading in wound edge cells [Coomber and Gotlieb, 1990]. It is also not clear what pathways or mechanisms are employed during bFGF induced cell signaling which could lead to cell migration and subsequent monolayer regeneration.

However, an intriguing possibility is suggested by the recent observation that EGF receptor contains an actin binding site on its intracellular domain [den Hartigh et al., 1992]. While it remains to be seen whether this is a feature of other catalytic growth factor receptors, such actin binding may be involved in modulating growth factor receptor/cytoskeleton interactions [den Hartigh et al., 1992]. Interaction of bFGF with its receptor could therefore potentially transmit or transduce cellular signals via the actin cytoskeleton. Such activity could be a consequence or a reflection of actin filament rearrangements seen in spreading wound edge cells of this present study.

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REFERENCES

Baldin V, Roman A-M, Bosc-Bierne I, Amalric F, Bouche G (1990): Translocation of bFGF to the nucleus is G_1 phase cell cycle specific in bovine aortic endothelial cells. EMBO J 9:1511–1517.

Bugler B, Amalric F, Prats H (1991): Alternative initiation of translation determines cytoplasmic or nuclear localization of basic fibroblast growth factor. Mol Cell Biol 11:573–577.

Cardinali M, Sartor O, Robbins KC (1992): Suramin, an experimental chemotherapeutic drug, activates the receptor for epidermal growth factor and promotes growth of certain malignant cells. J Clin Invest 89:1242–1247.

Cattini PA, Nickel B, Bock M, Kardami E (1991): Immunolocalization of basic fibroblast growth factor (bFGF) in growing and growth inhibited placental cells: A possible role for bFGF in placental cell development. Placenta 12:341–352.

Coomber BL (1991): Cytoskeleton in TGF-β- and bFGF-modulated endothelial monolayer repair. Exp Cell Res 194:42–47.

Coomber BL, Gotlieb AI (1990): In vitro endothelial wound repair: Interaction of cell migration and proliferation. Arteriosclerosis 10:215–222.

Cunningham CC, Gorlin JB, Kwiatkowski DJ, Hartwig JH, Janmey PA, Byers HR, Stossel TP (1992): Actin binding protein requirements for cortical stability and efficient locomotion. Science 255:325–327.

Dell'Era P, Presta M, Ragnotti G (1991): Nuclear localization of endogenous basic fibroblast growth factor in cultured endothelial cells. Exp Cell Res 192:505–510.

den Hartigh JC, van Bergen en Henegouwen PMP, Verkleij AJ, Boonstra J (1992): The EGF receptor is an actin-binding protein. J Cell Biol 119:349–355.

Eisenberger MA, Fontana JA (1992): Suramin, an active nonhormonal cytotoxic drug for treatment of prostate cancer: Compelling reasons for testing in patients with hormone-refractory breast cancer. J Natl Cancer Inst 84:3–5.

Euteneuer U, Schliwa M (1992): Mechanism of centrosome positioning during the wound response in BSC-1 cells. J Cell Biol 116:1157–1166.

Flaumenhaft R, Moscatelli D, Saksela O, Rifkin DB (1989): Role of extracellular matrix in the activation of basic fibroblast growth factor: Matrix as a source of growth factor for long-term stimulation of plasminogen activator production and DNA synthesis. J Cell Physiol 140:75–81.

Gotlieb AI (1990): The endothelial cytoskeleton: Organization in normal and regenerating endothelium. Toxicol Pathol 18:603–617.

Leslie RJ, Saxton WM, Mitchison TJ, Neighbors B, Salmon ED, McIntosh JR (1984): Assembly properties of fluorescein-labelled tubulin in vitro before and after fluorescence bleaching. J Cell Biol 99:2146–2156.

Lopez Lopez R, Peters GJ, van Loenen AC, Pizao PE, van Rijswijk REN, Wagstaff J, Pinedo HM (1992): The effects of schedule, protein binding and growth factors on the activity of suramin. Int J Cancer 51:921–926.

McNeil PL, Muthukrishnan L, Warder E, D'Amore PA (1989) Growth factors are released by mechanically wounded endothelial cells J Cell Biol 109 811–822

Mignatti P, Morimoto T, Rifkin DB (1991) Basic fibroblast growth factor released by single, isolated cells stimulates their migration in an autocrine manner Proc Natl Acad Sci USA 88 11007–11011

Mignatti P, Morimoto T, Rifkin DB (1992) Basic fibroblast growth factor, a protein devoid of secretory signal sequence, is released by cells via a pathway independent of the endoplasmic reticulum–golgi complex J Cell Physiol 151 81–93

Minniti CP, Maggi M, Helman LJ (1992) Suramin inhibits the growth of human rhabdomyosarcoma by interrupting the insulin-like growth factor II autocrine growth loop Cancer Res 52 1830–1835

Muthukrishnan L, Warder E, McNeil PA (1991) Basic fibroblast growth factor is efficiently released from a cytolsolic storage site through plasma membrane disruptions of endothelial cells J Cell Physiol 148 1–16

Pagano RE, Sepanski M, Martin OC (1989) Molecular trapping of a fluorescent ceramide analogue at the Golgi apparatus of fixed cells Interaction with endogenous lipids provides a trans-Golgi marker for both light and electron microscopy J Cell Biol 109 2067–2079

Pesenti E, Sola F, Mongelli N, Grandi M, Spreafico F (1992) Suramin prevents neovascularization and tumor growth through blocking of basic fibroblast growth factor activity Br J Cancer 66 367–372

Powell PP, Klagsbrun M (1991) Three forms of rat basic fibroblast growth factor are made from a single mRNA and localized to the nucleus J Cell Physiol 148 68–74

Presta M, Maier JAM, Rusnati M, Ragnotti G (1989) Basic fibroblast growth factor is released from endothelial extracellular matrix as a biologically active form J Cell Physiol 140 68–74

Ruoslahti E, Yamaguchi Y (1991) Proteoglycans as modulators of growth factor activities Cell 64 867–869

Sato Y, Abe M, Takaki R (1990) Platelet factor 4 blocks the binding of basic fibroblast growth factor to the receptor and inhibits the spontaneous migration of vascular endothelial cells Biochem Biophys Res Commun 172 595–600

Sato Y, Rifkin DB (1988) Autocrine activities of basic fibroblast growth factor Regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis J Cell Biol 107 1199–1205

Schwartz SM, Selden SC III, Bowman P (1979) Growth control in aortic endothelium at wound edges In Sato G, Ross R (eds) "Hormones and Cell Culture," Vol 6B New York Cold Spring Harbor Laboratory Press, pp 593–610

Singer SJ, Kupfer A (1986) The directed migration of eukaryotic cells Annu Rev Cell Biol 2 337–365

Theriot JA, Mitchison TJ (1992) Comparison of actin and cell surface dynamics in motile fibroblasts J Cell Biol 118 367–377

Tsuboi R, Rifkin DB (1990) Biomodal relationship between invasion of the amniotic membrane and plasminogen activator activity Int J Cancer 46 56–60

Tsuboi R, Sato Y, Rifkin DB (1990) Correlation of cell migration, cell invasion, receptor number, proteinase production, and basic fibroblast growth factor levels in endothelial cells J Cell Biol 110 511–517

Wade TP, Kasid A, Stein CA, LaRocca RV, Sargent ER, Gomella LG, Myers CE, Linehan WM (1992) Suramin interference with transforming growth factor-β inhibition of human renal cell carcinoma in culture J Surg Res 53 195–198