Fibroblast Growth Factor-2 Selectively Stimulates Angiogenesis of Small Vessels in Arterial Tree

Patricia Parsons-Wingerter, Katherine E. Elliott, John I. Clark, Andrew G. Farr

Abstract—There is a critical need for quantifiable models of angiogenesis in vivo, and in general, differential effects of angiogenic regulators on vascular morphology have not been measured. Because the potent angiogenic stimulators fibroblast growth factor (FGF)-2 (basic FGF) and vascular endothelial growth factor (VEGF) are reported to stimulate angiogenesis through distinct signaling pathways, we hypothesized that FGF-2 stimulates vascular morphology differently than does VEGF and that stimulation of angiogenesis by FGF-2 is directly correlated to FGF receptor density. FGF-2 was applied at embryonic day 7 (E7), E8, or E9 to the quail chorioallantoic membrane (CAM); subsequent response of the arterial tree was measured by the fractal dimension (Df), a mathematical descriptor of complex spatial patterns, and by several generational branching parameters that included vessel length density (Ll). After application of FGF-2 at E7, arterial density increased according to Df as a direct function of increasing FGF-2 concentration, and FGF-2 stimulated the growth of small vessels, but not of large vessels, according to Ll, and other branching parameters. For untreated control specimens at E7, Ll of small vessels and Df were 11.1±1.6 cm⁻¹ and 1.38±0.01, respectively; at E8, after treatment with 5 µg FGF-2/CAM for 24 hours, Ll of small vessels and Df increased respectively to 22.8±0.7 cm⁻¹ and 1.49±0.02 compared with 16.3±0.9 cm⁻¹ and 1.43±0.02 for PBS-treated control specimens. Application of FGF-2 at E8 and E9 did not significantly increase arterial density. By immunohistochemistry, the expression of 4 high-affinity tyrosine kinase FGF receptors was significantly expressed at E7, when CAM vasculature responded strongly to FGF-2 stimulation, but FGF receptor expression decreased throughout the CAM by E8, when vascular response to FGF-2 was negligible. In conclusion, the “fingerprint” vascular pattern elicited by FGF-2 was distinct from vascular patterns induced by other angiogenic regulators that included VEGF₁₆₅, transforming growth factor-β₁, and angiostatin. (Arterioscler Thromb Vasc Biol. 2000;20:1250-1256.)

Key Words: quail ■ chorioallantoic membrane ■ fractal dimension ■ fibroblast growth factor receptors ■ complexity

Fibroblast growth factor (FGF)-2, also known as basic FGF, is a powerful stimulator of angiogenesis in vivo and a pleiotropic regulator of the proliferation, migration, differentiation, and survival of many cell types in vitro, including endothelial cells, smooth muscle cells, and pericytes.₁⁻¹⁰ The FGFs, a heparin-binding family of structurally related cytokines, are widely expressed during embryonic development and normal wound healing, as well as in such angioproliferative diseases as cancer and diabetes. FGF-2 is expressed in at least 4 isoforms: an 18-kDa form (found at the cell surface, in the cytoplasm, and in the extracellular matrix) and the high molecular weight forms of 21.5, 22, and 24 kDa (all 3 associated predominantly with the nucleus). The 4 high-affinity FGF receptors (FGFRs) constitute a tyrosine kinase receptor family located at the cell surface.⁵⁻⁷,¹₀⁻¹₃ At least 1 alternatively spliced variant from FGFR1 through FGFR4 recognizes FGF-2, with the possible exception of FGFR4, for which ligand specificity is not yet resolved. Because of the difficulty of imaging 3D vascular trees embedded within opaque tissue, there is a lack of observation and quantification of the relative effects of FGF-2 and other angiogenic regulators on specific aspects of vascular morphology in vivo. The study of receptor-ligand interactions in such 3D vascular trees is also difficult. It has been reported that in the chicken chorioallantoic membrane (CAM), FGF-2 stimulated the growth of small vessels, whereas transforming growth factor (TGF)-β₁ stimulated the growth of large vessels.¹⁴ However, this single observation of the effect of FGF-2 on vascular form was qualitative.

In a comparative review of quantitative assays of angiogenesis, Jain et al¹⁵ concluded that there were no established assays of angiogenesis in vivo that were reasonably convenient with respect to performance and quantification. However, we recently described a model of angiogenesis in the quail CAM, in which the quasi-2D vasculature of the CAM is easily and uniformly exposed to angiogenic regulators in solution.¹⁶ The basal rate of angiogenesis in the rapidly developing transparent CAM responds to stimulation and inhibition, and resulting changes in the complex vascular
pattern are measured by fractal/generational branching morphometry.

An intriguing advantage of the uniform stimulation of angiogenesis within the quail CAM is the revelation of vascular pattern: each major regulator of angiogenesis applied to the CAM, including TGF-β, angiotatin, and vascular endothelial growth factor (VEGF)α5 (P.P.-W. et al, unpublished data, 2000), has elicited a “fingerprint” vascular pattern that is spatiotemporally unique and is well correlated with previously published angiogenic activities. In the present study, the response of vascular pattern to exogenous FGF-2 was quantified in the CAM arterial tree by the fractal dimension (Df) and by several generational branching parameters. Angiogenic stimulation by FGF-2 was also correlated with the kinetics of expression of FGFR1 through FGFR4.

Methods

Embryonic culture, assay, mounting, imaging, and fractal/grid intersection analysis have been described previously.16

Culture, Assay, and Mounting

Fertilized eggs of Japanese quail (Coturnix coturnix japonica, Boyd’s Bird Co, Pullman, Wash) were incubated at 37°C under ambient atmosphere, cracked at embryonic day 3 (E3) after incubation of the eggs for 56 hours, and cultured further at 37°C in Petri dishes (area of well bottom, 10 cm²). Recombinant human FGF-2 (154 amino acids [–1 methionine], FIBLAST, product code P8504) was the generous gift of Dr Judith Abraham (Scios, Mountain View, Calif). At E7, E8, or E9, FGF-2 was mixed to the desired concentration in prewarmed PBS, and 0.5 mL of solution was immediately applied in drops to the surface of each CAM. The embryos were incubated further at 37°C for 24 or 48 hours, at which time they were fixed in 4% paraformaldehyde/2% glutaraldehyde/PBS. If the diameter of an immature avian epithelial cell is assumed to be 13 μm, an application of FGF-2 at 10 μg/mL represents a stimulus of 0.5 and 0.25 pg per cell at E7 and E9, respectively, when calculated for a CAM of known surface area en face.16 The calculation further assumes that the CAM consists of 5 cellular layers, i.e., the 2 layers of the epithelial bilayers, the chorion, and the allantois, separated by an intervening mesenchymal layer. In general, however, FGF-2 treatment of the CAM is expressed as a mass amount (micrograms per CAM) rather than as a concentration (micrograms per milliliter), because the embryos appear to absorb the PBS solution almost immediately. Thus, the perturbant amount, rather than the concentration, appears to be the important parameter.

Imaging

Digital images of terminal arterial vessels from the middle region of the CAM were acquired in gray scale at a total magnification of ×10 and resolution of 13 μm per pixel, processed to black/white, and skeletonized.16 There was no significant increase in vessel density at a total magnification of ×20 compared with ×10.

Fractal Dimension and Grid Intersection

A total of 126 CAM specimens were analyzed; additional specimens provided quantitative confirmation of the analytical results. Each data point represents the mean ±SD of a minimum of quadruplicate CAMs from at least 2 independent experiments (with the exception of triplicate CAMs from embryos that were treated with 40 μg FGF-2 per milliliter and fixed at E10). Nine independent experiments were performed, and a minimum of 2 PBS-treated control specimens per experiment was analyzed for each time point (eg, 10 specimens were analyzed for PBS-treated control specimens at E8). Confidence limits (probability value) were calculated by a 2-tailed heteroscedastic Student t test.

Df, a statistical descriptor of space-filling pattern and density, necessarily varies from 1 to 2 for 2D binary fractal images. Df was estimated for skeletal images with a computer program16 implementing the method of box counting, for which least squares regression analyses of the data consistently produced a linear fit with a confidence level (r²) ≥0.97. We observed previously that fractal analysis was more sensitive to change in vascular pattern in skeletal images than in binary images.16 The skeletal image of a vascular tree is a direct representation of total vessel length,16,17 Grid intersection, a second statistical method that correlates positively with the fractal dimension,16 was used to confirm the results for Df at a grid size of 32 pixels.

Generational Analysis of Vascular Branching

Vessels were classified into their respective branching generations according to their proportional decrease in vessel diameter,13 because the strongest correlate to vessel generation in highly branched vascular trees is vessel diameter, not vessel length or branching angle.18–20 The largest arterial tree was extracted from a binary image, and the single parent vessel (also the vessel of largest diameter) was designated as the first generation (G1). If internal cross-sectional area is conserved, as is usually assumed for branching vessels because of the conservation of blood flow, the theoretical inner diameter of the 2 equivalent daughter vessels branching from a parent vessel is 71% of the inner diameter of the parent vessel. Vessels of G3 or greater were lumped as a single generational parameter (G≥3), because of limiting resolution for accurate measurement of vessel diameter.

Parameters of vessel length density (L), vessel area density (A), and vessel number density (N) for each branching generation G, through G≥3 were measured with a computer program (VESGEN, written by P.P.-W.). Average vessel diameter (Dv) was calculated as Dv = A/L per branching generation. We use the symbols A, L, and Dv to denote these parameters when they do not refer to specific generations, whereas, for example, Dv1, Dv2, and Dv3 denote Dv with respect to the specific generations G1, G2, and G3, respectively. VESGEN compares binary and skeletal images of the isolated arterial tree by using 8-pixel neighborhood connectivity and the binary morphological operators of the Image Processing Toolbox of Matlab software (Mathworks). It is also important to note that binary and skeletal images are direct representations of total vessel area (A(ter)all) and total vessel length (L(ter)all) for all branching generations G, through G≥3, respectively, as imaged in the 2D plane of the CAM en face.

The boundary of the normalizing area was determined by bisection of the distance between the arterial tree and neighboring arteries. N, L, and A (but not Dv) were expressed as density functions by normalization to the CAM surface area that is occupied by the extracted arterial tree. For analysis by VESGEN, images from 4 specimens for each of the following 6 data points were taken from the data set used for fractal analysis: (1) E7 (0 hours, no FGF-2), (2) and 3) E8 (24 hours with and without 5 μg FGF-2/CAM), (4) and 5) E9 (48 hours with and without 5 μg FGF-2/CAM), and (6) E10 (72 hours, no FGF-2).

Immunohistochemistry

Antibodies recognizing peptide sequences within the intracelluar domains to FGFR1 through FGFR4 (all rabbit polyclonal IgG, sc/121 through sc/124, Santa Cruz Biotechnology) were applied with and without blocking peptides (sc/121-P through sc/124-P, Santa Cruz Biotechnology) to CAM specimens (not treated with either PBS or FGF-2) by conventional whole-mount immunohistochemistry (IHC).21 Although receptors homologous to FGFR1 through FGFR4 have not yet been identified in the quail, the immunogenic peptide sequences used for the present study are in general highly conserved (ie, the sequences are found in FGF-like receptors in the chicken, frog, and newt, as well as in the human, rat, and mouse). The QH-1 monoclonal antibody (Developmental Studies Hybridoma Bank) specifically recognizes quail vasculature and an unidentified hematoietic precursor cell.22,23 The CAMs were fixed for 48 hours at 4°C at E7 by 4% paraformaldehyde/PBS and at E8 by 2% paraformaldehyde/PBS. Cross-reactivity among primary and secondary antibodies (fluorescein-conjugated goat IgG fraction to rabbit immunglobulins, No. 55652, Cappel, and rhodamine-conjugated goat IgG fraction to mouse IgG, No. 55527, Cappel), as well as to irrelevant rabbit IgG (substituted for primary antibody), was low. Digital images of CAM specimens were acquired at ×100 and ×400 total magnification.
Results

FGF-2 Selectively Stimulates Angiogenesis of Small Vessels

According to visual inspection of binary images (Figure 1), FGF-2 strongly stimulated arterial density when applied for 24 or 48 hours to specimens at E7, relative to PBS-treated control specimens (Figure 1A, 1B, 1E, and 1F). Inspection of the images also suggested that FGF-2 selectively increased the density of smaller terminal arteries but not of larger arteries. However, FGF-2 did not appear to stimulate arterial growth significantly when applied for 24 hours at E8 or E9 (Figure 1C, 1D, 1G, and 1H). As measured by Df (Figure 2A and 2B), angiogenesis increased as a direct function of increasing amounts of FGF-2 from 0.004 to 5 μg per CAM, when FGF-2 was applied at E7 for 24 or 48 hours. For untreated control specimens at E7 (0 hours), Df was 1.38±0.01. The increase in vascular density in response to FGF-2 was maximal at 5 μg per CAM, for which Df was 1.49±0.02 after 24 hours compared with Df of 1.43±0.02 for (PBS-treated) control specimens, and Df was 1.55±0.01 in response to FGF-2 after 48 hours compared with 1.50±0.02 for control specimens. Stimulation of arterial density by FGF-2 was distinguished from control specimens at a confidence level of >90% for amounts as low as 0.1 μg per CAM after 24 hours and 2.5 μg per CAM after 48 hours.

In contrast, stimulation of angiogenesis by FGF-2 was considerably less when FGF-2 was applied at E8 or E9, even at amounts as high as 20 μg per CAM (Figure 2C and 2D). By Df, the largest increase in arterial density after application of FGF-2 at E8 and E9 for 24 hours was 1.52±0.01 for specimens treated with 1.25 μg FGF-2/CAM at E8 compared with 1.49±0.02 for control specimens (Figure 2C). However, response to 10 μg FGF-2/CAM by Df was variable at E10 after application at E9 for 24 hours, suggesting that there may have been some stimulatory activity by FGF-2 under these experimental conditions.

All results for Df (Figure 2) were confirmed by grid intersection (ρv, data not shown). For untreated control specimens at E7, ρv was 648±51/cm². In response to treatment with 5 μg FGF-2/CAM at E7, ρv increased after 24 hours (at E8) to 1090±96/cm² compared with 835±85/cm² for control specimens, and after 48 hours (at E9), ρv increased to 1420±33/cm² compared with 1118±113/cm² for control specimens.
According to morphological parameters of the arterial tree for branching generations G\textsubscript{1} through G\textsubscript{5}, the application of FGF-2 at E7 for 24 hours or 48 hours stimulated the growth of small arteries but not of large arteries (Figure 3). For untreated control specimens at E7 (0 hours), the length density of small arteries (L\textsubscript{v5}) was 11.1±1.6 cm\textsuperscript{-1}. After treatment at E7 with 5 mg FGF-2/CAM, L\textsubscript{v5} increased to 22.8±0.7 cm\textsuperscript{-1} after 24 hours (at E8) compared with 16.3±0.9 cm\textsuperscript{-1} for control specimens and to 30.7±2.4 cm\textsuperscript{-1} after 48 hours (at E9) compared with 25.0±0.9 cm\textsuperscript{-1} for control specimens (Figure 3A). The length density of large arteries (L\textsubscript{v1-4}), which for untreated control specimens at E7 was 5.9±1.1 cm\textsuperscript{-1}, was not affected significantly by FGF-2. After treatment at E7 with 5 mg FGF-2/CAM, L\textsubscript{v1-4} was 6.3±1.1 cm\textsuperscript{-1} after 24 hours (at E8) compared with 6.2±0.4 cm\textsuperscript{-1} for control specimens, and L\textsubscript{v1-4} was 7.1±1.0 cm\textsuperscript{-1} after 48 hours (at E9) compared with 6.2±0.3 cm\textsuperscript{-1} for control specimens (Figure 3A).

Results for A\textsubscript{v} (Figure 3B) and N\textsubscript{v} (Figure 3C and 3D) correlated positively with those for L\textsubscript{v}. For example, for untreated control specimens at E7, the number density of small arteries (N\textsubscript{v5}) was 529±53 cm\textsuperscript{-2}. After treatment at E7 with 5 mg FGF-2/CAM, N\textsubscript{v5} increased to 882±74 cm\textsuperscript{-2} after 24 hours (at E8) compared with 663±141 cm\textsuperscript{-2} for control specimens and to 1318±207 cm\textsuperscript{-2} after 48 hours (at E9) compared with 904±135 cm\textsuperscript{-2} for control specimens (Figure 3A). For N\textsubscript{v5}, confidence limits for specimens treated at E7 for 48 hours (until E9) with 5 mg FGF-2/CAM were 95% at E8 and 98% at E9 relative to control specimens. As for L\textsubscript{v1-4}, the number density of large arteries (N\textsubscript{v1-4}), which for untreated control specimens at E7 was 36.2±8.5 cm\textsuperscript{-2}, was not affected significantly by FGF-2. In response to treatment at E7 with 5 mg FGF-2/CAM, N\textsubscript{v1-4} was 32.0±6.5 cm\textsuperscript{-2} after 24 hours (at E8) compared with 34.8±3.5 cm\textsuperscript{-2} for control specimens, and N\textsubscript{v1-4} was 33.2±4.0 cm\textsuperscript{-2} after 48 hours (at E9) compared with 34.6±7.2 cm\textsuperscript{-2} for control specimens (Figure 3A).

The average length per smaller vessel (L\textsubscript{v5}/N\textsubscript{v5}) was relatively unaffected by FGF-2. For example, for specimens treated at E7 for 24 hours (until E8) with 5 mg FGF-2/CAM, L\textsubscript{v5}/N\textsubscript{v5} was 258±31 mm compared with 246±31 mm for control specimens. D\textsubscript{v} was also not affected significantly by FGF-2 (data not shown; see Figure 1). For specimens treated at E7 for 24 hours (until E8) with 5 mg FGF-2/CAM, D\textsubscript{v5} was 150±31 and 49±4 mm, respectively, compared with 171±26 and 47±4 mm for control specimens.

**Increased Expression of FGF Receptors Coincides With Angiogenic Stimulation by FGF-2**

By fluorescent whole-mount IHC of normal untreated CAM specimens at magnifications of ×400 (Figure 4) and ×100 (data not shown), immunological staining for high-affinity tyrosine kinase receptors FGFR1 through FGFR4 was strongly positive throughout the CAM at E7 (the time at
which arteries responded most vigorously to FGF-2 stimulation, Figures 1 to 3). Staining for FGFR1 was bright and punctate throughout the CAM. In contrast, staining for FGFR2 was very strong but diffuse. Morphology and distribution of FGFR3 and FGFR4 combined the bright punctate pattern associated with FGFR1 and the diffuse localization of FGFR2, expressed at lower levels of intensity. However, the expression of FGFR1 through FGFR4, particularly that of FGFR2, was significantly reduced at E8 compared with the expression of these receptors at E7.

By counterstaining for vasculature with the QH1 antibody (Figure I, which can be found online at http://atvb.ahajournals.org), colocalization of FGFR1 through FGFR4 with blood vessels was often strong but variable (see, eg, Figure 4A, 4B, and 4D and Figure 1A, IB, and ID versus Figure 4C and Figure IC). Blocking of immunological reactivity with FGFR1 through FGFR4 by the immunogenic peptides was virtually complete, and cross-reactivity with secondary antibody was low (data not shown).

Discussion

Stimulation of angiogenesis in the quail CAM by FGF-2 was manifested as 3 significant well-correlated morphological events that were quantified by fractal/generational branching morphometry and further characterized by fluorescent IHC. First, by Df and generational branching analysis of Lv, Av, and Nv, arterial density increased with increasing concentration of FGF-2, until maximal arterial density was reached at 5 μg FGF-2/CAM. Second, by Lv, Av, and Nv, FGF-2 selectively stimulated the growth of small but not large arteries. This result was further confirmed by visual inspection of the images and is in agreement with the qualitative report by Yang and Moses.14 Third, as indicated by IHC, the rapidly developing arterial tree responded strongly to stimulation by FGF-2 only when FGFRs 1 through 4 (especially FGFR2) were expressed at high densities throughout the CAM at E7 but not when FGFRs 1 through 4 were expressed at low densities at E8.

The importance of specific morphological changes elicited by FGF-2 is underscored by our recent observation that major stimulators and inhibitors of angiogenesis induce distinct “fingerprint” patterns of vascular morphology when applied to the quail CAM. The angiogenic inhibitors TGF-β1 17 and angiostatin16 induced changes in the vascular pattern of the angiogenic quail CAM that were distinctly different from each other. VEGF165 increased the diameters and permeability of large arteries when applied at E7 at high concentration (P.P.-W. et al, unpublished data, 1999). At low concentrations, VEGF165, like FGF-2, stimulated the angiogenesis of smaller arteries. Our quantified studies of perturbed vascular morphology induced by FGF-2 and VEGF165 suggest that FGF-2 may act as a powerful but simple (ie, unimodal) regulator of vascular maturation, whereas VEGF may act as a complex (ie, multimodal) vascular regulator. The increases in all morphological parameters induced by FGF-2 correlated positively with the normal maturation increases in these parameters displayed by control specimens (with the exception of Nv1-4 in control specimens at E10).

Our IHC results (Figure 4) indicate that stimulation of angiogenesis in the arterial tree of the quail CAM by FGF-2 is regulated by the spatial and temporal expression of FGFR1 through FGFR4. Arterial growth and density responded most strongly to the application of FGF-2 at E7, when FGFRs 1 through 4 were present at high density in the CAM and on vessels of the arterial tree; response to FGF-2 declined at E8, when FGFR density decreased. These IHC results correlate with our previous findings that angiogenesis in the CAM occurs most rapidly (and at a linear rate) from E7 to E10, after which angiogenesis ceases.16 The decreased availability of FGFR1 through FGFR4 throughout the CAM may be one of the mechanisms that switch off the angiogenic process in the CAM by E10.

The antibodies used in our IHC are specific to peptide sequences within intracellular domains of FGFR1 through FGFR4, which are more highly conserved among the many splice variants of the FGFR family than the external IgG-like
domains. At least 1 alternatively spliced variant per FGFR1 through FGFR4 recognizes the FGF-2 ligand (with the possible exception of FGFR4). Specificity of the immunogenic peptide sequences to FGFR1 through FGFR4 in the antibodies may not be absolute. Nonetheless, the antibodies are peptide specific, and patterns of FGFR downregulation appear plausible, given vascular response to the kinetics of FGF-2 application. In situ hybridization would confer greater specificity with respect to FGFR1 through FGFR4 but would not necessarily correlate with protein localization.

The targeted disruption of the FGF-2 gene and the transgenic overexpression of FGF-2 in mice have resulted in rather subtle vascular phenotypes. For example, null expression of FGF-2 was associated with low blood pressure but normal vascular development, with decreased vessel permeability but no difference in neovascularization subsequent to laser-induced choroidal injury, and with no decrease in neovascularization of the hypoxic retina. After the murine wound-healing model, but neovascularization did not increase in the hypoxic retina. These rather modest mutational results with respect to neovascularization were surprising, because in general, exogenous FGF-2 strongly increases angiogenesis in vivo. For example, a single intracoronary bolus of FGF-2 initiated angiogenesis and coronary perfusion in an ischemic porcine model, and exogenous immunological antagonism of FGF-2 altered carotid arterial remodeling in the mouse. The apparent contradiction between strong and weak angiogenic regulation by exogenous and endogenously altered FGF-2, respectively, has been attributed to the possible redundancy of the FGFR/FGF receptor-ligand system. It appears that 18 distinct FGF genes have been identified, which are expressed as numerous alternatively spliced isoforms. FGFRs and receptor-ligand binding specificities also display a similar combinatorial diversity. Angiogenic regulation by endogenous FGF-2 and other FGFs is perhaps controlled in part by the kinetics of FGFR expression at the cell surface and the targeted release of soluble FGFR ectodomains, the extracellular domains of FGFRs, to the extracellular matrix and the blood. For recent advances in the regulatory role of ectodomain shedding, see Reference 34.)

Recently, structural and biochemical binding studies of FGF-2 and other FGFs demonstrated that residues of the primary binding site within FGF-2 to the extracellular immunoglobulin II domains of FGFRs are conserved throughout the FGF family, whereas residues of a secondary binding site within FGF-2 and other FGF family members to the extracellular immunoglobulin III domains of FGFRs are variable.

A critical role in angiogenesis for VEGF, the highly endothelial cell-specific cytokine, has been established in recent years, because lack of 1 of the 2 VEGF alleles results in embryonic death that is due to defective cardiovascular development. But VEGF is apparently not the sole stimulator of angiogenesis; VEGF antagonists inhibited neovascularization in vivo by only ~50%, and FGF-2 was required for the upregulation of VEGF before vasculogenesis in the quail blastodisc. The potent angiogenic stimulators FGF-2 and VEGF appear to stimulate angiogenesis through distinct signaling pathways mediated by the transmembrane tyrosine kinase integrin receptors that are specific to each regulator, although cross talk between signaling by FGF-2 and VEGF has been reported. In the murine retina and chicken CAM, stimulation of angiogenesis by FGF-2 or by VEGF was associated with signaling by the aβ or the aβ integrins, respectively. In migrating human endothelial cells, FGF-2 stimulated chemokinesis (the component for rate of random displacement in cell movement), whereas VEGF stimulated chemotaxis (the directional component of cell movement). After exogenous delivery of FGF-2 or VEGF to a collagen window applied to the mouse cranium or skin, initial angiogenic response was controlled by the growth factors, but long-term physiological properties of the new vessels were most dependent on the local tissue environment and independent of the initial angiogenic stimulus. Interestingly, overall inhibition of tyrosine kinase receptors, the high-affinity receptor types for both FGF-2 and VEGF, almost completely inhibited neovascularization in a hypoxic model in vivo.

In conclusion, major angiogenic regulators that have elicited specific patterns of perturbed vascular morphology in the quail CAM include the stimulators FGF-2 and VEGF, (P.P.-W. et al, unpublished data, 2000) and the inhibitors angiostatin and TGF-β. Quantification of angiogenesis in the quail CAM supports the inference of dominant regulatory mechanisms from spatiotemporal alterations in vascular pattern, which can then be studied in other models of angiogenesis that specifically test the proposed mechanisms. Thus, the quail CAM model of angiogenesis can provide insight into emergent biological properties, ie, fundamentally important nonlinear properties of highly cooperative processes, such as angiogenesis, that cannot be revealed by reductionist studies alone.

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FIG. 5 (Online Only)