Case Report

Fibroblast growth factor 2 and the transcription factor Egr-1 localise to endothelial cell microvascular channels in human coronary artery occlusion

A 69-year old male with angina, hypertension, hyperlipidemia and Type II diabetes mellitus underwent percutaneous coronary intervention (PCI) to a 1–2 month old total occlusion of the right coronary artery (RCA) (Fig. 1A-C). In an uncomplicated and successful procedure, a distal protection device (Filter Wire EX, Boston Scientific, Natick, MA, USA) was used to minimise peri-procedural distal embolisation into viable myocardium (Fig. 1B). Immunohistopathology of the recovered material forms this report.

Following the procedure, material from the device was placed in 10% neutral buffered formalin, and paraffin embedded. Haematoxylin and Eosin and Masson's Trichrome stains were performed on 5 µm serial sections. In addition, the following were examined using specific antibodies: the transcription factors early growth response factor-1 (Egr-1) and c-jun (Santa Cruz Biotechnology, Santa Cruz, CA, USA); the growth factors Fibroblast Growth Factor 2 (FGF-2) and Vascular Endothelial Growth Factor (VEGF) (Santa Cruz Biotechnology), Proliferating Cell Nuclear Antigen (PCNA) (Dako, Carpinteria, CA, USA), Tissue Factor (TF) (gift from Prof. Wolfram Ruf, Scripps Research Institute La Jolla, CA, USA) and the endothelial cell marker CD31 (Santa Cruz Biotechnology). Secondary antibodies were 3-amino-9-ethylcarbazole (AEC) for CD 31 and PCNA, and 3,3'-diaminobenzadine (DAB) for Egr-1, c-jun, FGF-2, TF and VEGF, respectively.

The tissue fragments measured up to 4.2 mm in length and up to 1.5 mm in diameter. They were composed of fibrin and a small amount of newly deposited collagenous connective tissue, together with neovascular channel formation throughout the tissue (Fig. 1D, E), suggestive of organising thrombus. The vascular channels were comprised of a single layer of endothelial cells, confirmed by positive staining for CD31 (Fig. 1F), which were PCNA positive (Fig. 1G) suggesting active proliferation. From the extent of neovascular channel formation, and the degree of proliferation, it is estimated this organising thrombus was several weeks old. This is consistent with the clinical evidence, which placed the occlusion at 1–2 months old on angiographic grounds. The cells also showed some positive staining for Egr-1 (predominantly nuclear) and more markedly positive staining for FGF-2 (mainly nuclear and cytoplasmic) (Fig. 1H), though not for c-jun, VEGF or TF (data not shown). There was minimal staining for Egr-1 or FGF-2 elsewhere in these lesions.

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Chronic total coronary occlusions remain a problematic area in cardiology, with limited available treatments (1). Totally occluded coronary arteries are known to contain multiple microvascular channels after 3 months (2), which may progress to recanalisation, providing alternative pathways for antegrade blood flow and symptom relief. However, the mechanisms by which these channels form are unknown, and little is known of the histology prior to 3 months.

These data demonstrate, for the first time, localisation of FGF-2 and Egr-1 protein to endothelial cells forming microvascular channels throughout organising thrombus, 1–2 months following human coronary artery occlusion.

FGF-2 (or basic FGF) is a polypeptide growth factor which modulates a number of cell functions including angiogenesis, vasculogenesis, cell proliferation and migration in a variety of cell types, but with particular emphasis on endothelial cells (3). It is a member of the larger fibroblast growth factor family, which contains over 20 structurally related polypeptides (3, 4). FGF-2 lacks a classical signal peptide, and although cellular release of the protein can occur via a number of other mechanisms, the actions of FGF-2 can occur via both intracellular and extracellular means (3, 4). These functions include activation of key transcription factor pathways. Egr-1 is a pleiotropic transcription factor, and a key upstream activator of a number of pathways mediating vascular cell proliferation, migration adhesion and coagulation (5).

The finding here of FGF-2 and Egr-1 up-regulation in endothelial cell microvascular channels within organising thrombus is intriguing. Clearly, co-localisation does not prove an association of function, and the limited tissue fragments available prevented more definitive co-localising studies. However, endothelial cell FGF-2 dependent Egr-1 transcription is known to occur in response to vascular injury (6). Similar FGF-2/Egr-1 dependent mechanisms have recently been invoked in the context of angiogenesis in neovascularisation and tumour growth (7). Given these observations, the present data suggest FGF-2 and Egr-1 may be involved in an endothelial gene regulatory circuit involved in the formation of these microvascular channels, and that similar processes may be involved in thrombus neovascularisation in the human coronary artery. The observation that these endothelial channels show PCNA positive staining also suggests that any FGF-2 and Egr-1 dependent processes are playing a role during the active, early proliferating phase of neovascularisation.

Whether FGF-2 regulates Egr-1 or vice-versa, or both, is not clear. Our data, nonetheless, indicate that coronary occlusions, rather than being an end-stage process, are active and undergo dynamic change, and suggest FGF-2 and Egr-1 may play a role in this process.

In contrast, VEGF and TF staining were negative. VEGF is a potent inducer of angiogenesis (8), and is known to play a role in
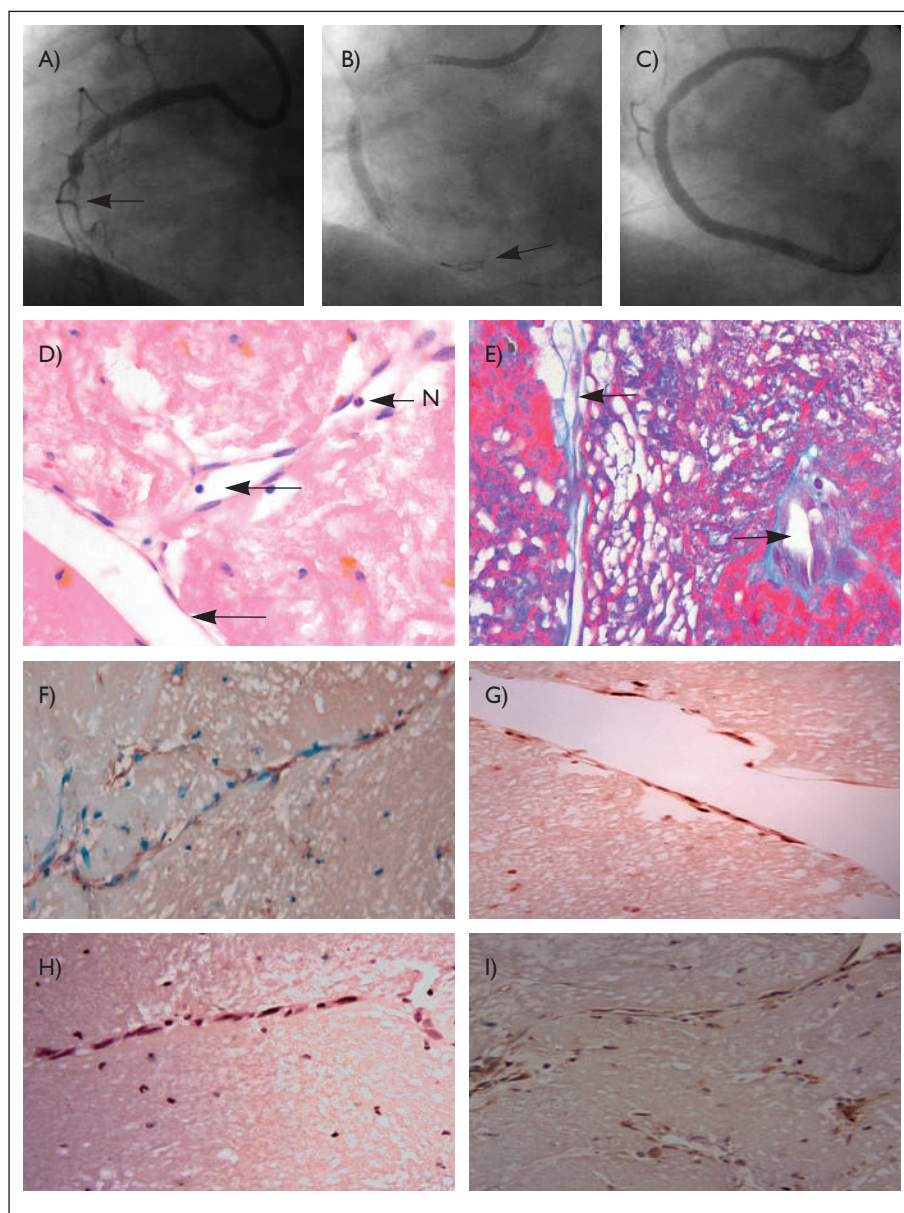
Figure 1: Immunohistopathology of material collected during percutaneous treatment of occluded right coronary artery. Coronary angiography, left anterior oblique (LAO) projection: A. Right coronary artery occlusion indicated at arrow; B. Balloon dilatation and stent deployment with distal protection device in-situ (arrow); C. Final angiographic result. Histopathology of material collected in distal protection device: D. Haematoxylin and Eosin stain (x 40) demonstrating neovascular channels (arrows) in the organising thrombus; an intravascular neutrophil is identifiable (labelled N); E. Masson's Trichrome stain (x 40) demonstrating neovascular channels (arrows) in longitudinal and cross section, in a matrix comprising a mixture of collagen fibres (blue) and fibrin (red). Immunohistochemical analysis of material collected: Neovasular channels within the organising thrombus stained using antibodies directed to: (F) CD31(4 µg/ml), (G) PCNA(10 µg/ml), (H) Egr-1(20 µg/ml), (I) FGF-2(2 µg/ml), magnification x 40. Hematoxylin counterstain for CD31 studies. Note predominantly nuclear staining for (G) PCNA and (H) egr-1; nuclear and cytoplasmic staining for (F) CD31 and (I) FGF-2.

recanalisation of venous thrombosis (9). Its role in recanalisation of arterial thrombus, and in arterial collateral formation however, is less clear (8, 10). Likewise, TF was not seen to be upregulated. TF expression is increased by Egr-1 in a number of contexts, and plays a role in thrombus formation (11). The negative findings here clearly do not preclude roles for VEGF or TF, but rather highlight the need for further examination of tissue obtained in this way, from similar patients.

These observations raise a number of additional issues. Firstly, given prior human experience of intracoronary FGF delivery (12), the potential for FGF to provide pro-angiogenic, therapeutic benefit for total occlusions may become a consideration. Second, the demonstration of large tissue fragments trapped in the distal protection device, suggests that significant distal embolisation was prevented and that these devices may have a place in the treatment of chronic coronary total occlusions. How often material collected in this way is useful for pa-

thologic studies however, is at present unknown. Finally, whilst the present data are limited to a single patient, such observations may prompt further investigation into local vascular reparative mechanisms following total coronary occlusion, potentially providing novel therapeutic options.

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