Increased myocardial expression of angiopoietin-2 in patients undergoing urgent surgical revascularization for acute coronary syndromes

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NP Fam, S Arab, F Billia, et al. Increased myocardial expression of angiopoietin-2 in patients undergoing urgent surgical revascularization for acute coronary syndromes. *Can J Cardiol* 2010;26(7):365-370.

BACKGROUND: Myocardial ischemia triggers the expression of multiple angiogenic factors including vascular endothelial growth factor and its receptors. However, vascular endothelial growth factor does not act in isolation.

OBJECTIVE: To identify other genes important in the angiogenic response to clinically relevant myocardial ischemia.

METHODS AND RESULTS: Paired intraoperative biopsies of ischemic and nonischemic myocardium were obtained from 12 patients with acute coronary syndromes (ACS) undergoing urgent coronary artery bypass graft surgery. Real-time polymerase chain reaction demonstrated significant upregulation of angiopoietin-2 (Ang-2) in ischemic myocardium, to a greater extent than other classical angiogenic factors. Microarray gene profiling identified Ang-2 to be among the top 10 differentially upregulated genes, in addition to genes involved in inflammation, cell signalling, remodelling and apoptosis.

CONCLUSIONS: The present document is the first report of microarray analysis of patients with ACS, and supports an important role for Ang-2 in the angiogenic response to severe ischemia in the human heart. Common gene expression patterns in ACS may provide opportunities for targeted pharmacological and cellular intervention.

Key Words: Angiogenesis; Molecular biology; Myocardial infarction; Revascularization

Angiogenesis is an important adaptive response to myocardial ischemia. In the context of cardiac ischemia, neovascularization can serve to salvage viable myocardium, thereby limiting infarct size and ventricular remodelling. Neovascularization of ischemic myocardium is mediated by multiple vascular growth factors and their receptors (1); paramount among these is vascular endothelial growth factor (VEGF). In addition to its potent direct angiogenic effects, VEGF released in response to tissue hypoxia and ischemia stimulates mobilization of bone marrow-derived stem cells to sites of tissue ischemia, and is a potent mediator of endothelial cell (EC) differentiation and proliferation. Increased expression of VEGF has been detected in both the peripheral blood (2) and myocardium (3) of patients with acute coronary syndromes (ACS), and presumably reflects enhanced myocardial angiogenesis.

The angiopoietins are ligands for an endothelial-selective receptor tyrosine kinase, Tie-2, and play an important and complementary role to the classical angiogenic factors such as VEGF. Angiopoietin-1 (Ang-1) is critical for neovascular stabilization and maturation, whereas angiopoietin-2 (Ang-2) antagonizes Ang-1 activation of Tie-2 receptors and facilitates the initiation of angiogenesis in response to VEGF (1). Recently, plasma levels of Ang-2 and Tie-2 were reported to be elevated in patients with ACS (4) and heart failure (5). However, elevated plasma levels of angiogenic growth factors may not accurately reflect local levels of growth factors in the heart. Elevation of Ang-2 levels has been reported

L'expression myocardique accrue de l'angiopoïétine-2 chez des patients subissant une revascularisation chirurgicale d'urgence secondaire à un syndrome coronarien aigu

HISTORIQUE : L'ischémie myocardique déclenche l'expression de facteurs angiogéniques multiples, y compris le facteur de croissance de l'endothélium vasculaire et ses récepteurs. Cependant, le facteur de croissance de l'endothélium vasculaire n'agit pas de manière isolée.

OBJECTIF : Dépister d'autres gènes importants dans la réponse angiogénique à une ischémie myocardique pertinente sur le plan clinique.

MÉTHODOLOGIE ET RÉSULTATS : Les chercheurs ont prélevé des biopsies intraopératoires appariées du myocarde ischémique et non ischémique chez 12 patients ayant un syndrome coronarien aigu (SCA) qui ont subi un pontage aortocoronarien d'urgence. La réaction en chaîne de la polymérase en temps réel a démontré une importante régulation à la hausse de l'angiopoïétine-2 (Ang-2) dans le myocarde ischémique, dans une plus grande mesure que les autres facteurs angiogéniques classiques. Le profil génique microréseau a permis d'établir que l'Ang-2 fait partie des dix principaux gènes à régulation différentielle à la hausse, en plus des gènes qui participent à l'inflammation, à la signalisation cellulaire, au remodelage et à l'apoptose.

CONCLUSIONS : Le présent document est le premier rapport d'une analyse microréseau de patients ayant un SCA et étaye le rôle important de l'Ang-2 dans la réponse angiogénique à une grave ischémie du cœur humain. Les motifs d'expression génique courants du SCA peuvent fournir des possibilités d'intervention pharmacologique et cellulaire ciblée.

in ischemic myocardium in the rat (6); however, to date, no studies have addressed the regulation of the expression of components of the angiopoietin system in human myocardium in response to ischemia.

Neovascularization is a complex process that involves the action of a multitude of gene products influencing angiogenesis, inflammation, cell death and survival. Although there is little doubt that candidate genes such as VEGF and, possibly, the angiopoietin family members, play an important role in the response to coronary ischemia, they do not act in isolation, but rather in concert with many other genes. Thus, changes in the overall pattern of gene expression are of critical importance for efficient collateral vessel formation within ischemic human myocardium. Microarray analysis has been used to profile global changes in human myocardial gene expression in a broad range of cardiovascular diseases including heart failure (7), dilated and hypertrophic cardiomyopathy (8), atrial fibrillation (9) and the response to left ventricular assist devices (7,10-12). However, to date, no studies have addressed gene expression profiles in ischemic human myocardium. Moreover, interindividual variability in gene expression related to differences in genetic influences, treatment and environmental factors often confound comparisons between different patients or patient groups.

In the present study, paired intraoperative biopsies were obtained from patients with ACS undergoing urgent coronary artery bypass graft (CABG) surgery, thus enabling the direct comparison of ischemic with

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Received for publication June 23, 2008. Accepted January 12, 2009

TABLE 1
Primer sequences used for real-time polymerase chain reaction

Gene	Sense primer sequence	Antisense primer sequence
<i>Ang-1</i>	5'-AAACTTCAACATCTGGAACATGTGA-3'	5'-ATCTGGGCCATCTCCGACTT-3'
<i>Ang-2</i>	5'-TCAGGACACACCACAAATGGCATC-3'	5'-CATCCTCACGTCGCTGAATAATT-3'
<i>Tie-1</i>	5'-CAGACGTGATCTGGAAGAGCAACG-3'	5'-GGTGGCTGCACATTGGGAGC-3'
<i>Tie-2</i>	5'-GGCTGGCCGCTACCTACTAA-3'	5'-GCTACTGAGAAATGATCCGTATGGTT-3'
<i>VEGF</i>	5'-GGGCAGAATCATCACGAAGTG-3'	5'-TCAGGGTACTCCTGGAAGATGTC-3'
<i>PIGF</i>	5'-ACGTGGAGCTGACGTTCTCTCAGC-3'	5'-TCCTCTTCCCCCTGCCCTTGG-3'
<i>Flt1</i>	5'-TGAGCGGCTCCCTTATGATGCC-3'	5'-ATGCCAAATGCTGATGCTTGAACC-3'
<i>Flk1</i>	5'-CAGCATCCAGTGGGCTGATGACC-3'	5'-TCGCAGGGATTCTGACACGCTCC-3'
<i>HIF1α</i>	5'-TTTGC GTGTGAGGAACTTCTG-3'	5'-AAAACCATCCAAGGCTTTCAAAT-3'
<i>eNOS</i>	5'-GTGGCTGTCTGCATGGACCT-3'	5'-CCACGATGGTGACTTTGGCT-3'
<i>Beta-actin</i>	5'-AGCCTCGCCTTTGCCGA-3'	5'-CTGGTGCTGGGGCG-3'

nonischemic myocardium in the same patient. We report a profound elevation in *Ang-2* messenger RNA (mRNA) and protein expression, exceeding that of other angiogenic genes and localized to regions of ischemia in human myocardium. Moreover, we report, for the first time, the application of microarray gene profiling among patients with ACS, confirming that *Ang-2* is among the top differentially regulated genes and identifying a number of other candidate genes in the complex response to clinically relevant myocardial ischemia.

METHODS

Patients and tissue sampling

A total of 20 adult patients undergoing CABG surgery were enrolled between 2001 and 2005, and provided informed consent for the study. Eight patients underwent elective surgery for stable angina and 12 underwent surgery for ACS (including unstable angina, non-ST elevation myocardial infarction [MI] and ST elevation MI). All ACS patients underwent CABG surgery within seven days of their index event. Patients were excluded if they were undergoing a combined procedure such as valve replacement and bypass grafting, had severe comorbid disease, New York Heart Association class IV heart failure or an ejection fraction of less than 20%, or were enrolled in a study using an experimental drug or procedure. Paired, partial thickness epicardial left ventricular biopsies were obtained intraoperatively from areas of presumed acute ischemia or infarction (based on tissue appearance and available electrocardiographic, echocardiographic, nuclear perfusion and angiographic data), and from an area judged to be relatively well perfused as a control. After biopsy, hemostasis was achieved with polypropylene sutures if needed. The protocol was approved by the St Michael's Hospital (Toronto, Ontario) Research Ethics Board.

Real-time polymerase chain reaction

Biopsy specimens from 17 patients (eight stable angina and nine ACS patients) were flash frozen in liquid nitrogen at -140°C . One-half of each specimen was used to isolate total RNA (TRNA) using the GenElute Total RNA Miniprep Kit (Sigma-Aldrich Co, Canada). First-strand complementary DNA synthesis was performed with 100 ng of TRNA using the Sensiscript RT kit (QIAGEN, Canada). Real-time polymerase chain reaction (PCR) for *Ang-1*, *Ang-2*, *Tie-1*, *Tie2*, vascular endothelial growth factor A (VEGFA), placental growth factor (PIGF), *Flt1*, *Flk1*, hypoxia inducible factor-1- α (HIF1 α), endothelial nitric oxide synthase (eNOS) and beta-actin was performed in triplicate using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, USA) and the QuantiTect SYBR Green PCR Kit (QIAGEN). The primer sequences (sense and antisense) used are listed in Table 1.

SDS 2.1 software (Applied Biosystems) was used for PCR data analysis. The specificity of the amplification product was confirmed by melting curve analysis and by detection of PCR products of correct size using agarose gel electrophoresis. Standard curves were generated for each gene using serial dilutions of known quantities of complementary DNA

template, allowing calculation of amplification efficiency for each primer pair. Relative quantification of gene expression was obtained by normalizing to alpha-actin. Gene expression in ischemic samples was expressed relative to nonischemic samples using the statistical relative expression software tool (REST) (13). Data from ischemic samples were expressed relative to nonischemic samples, which were arbitrarily assigned a value of 1; $P < 0.05$ was considered to be statistically significant.

Affymetrix GeneChip (Affymetrix, USA) hybridization and scanning
 TRNA was isolated from paired biopsy specimens in three patients with ACS. The quality of TRNA was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Canada). Only RNA with an optical density 260 nm/280 nm ratio of 1.99 to 2.0 was used. A total of seven hybridizations were performed on the Human HG-U133_PLUS2 GeneChip (Affymetrix), including six TRNAs from myocardial samples from the ischemic and nonischemic regions of three patients, and one reference TRNA (Stratagene, USA) that served as a universal control. Samples were prepared for hybridization according to standard Affymetrix instructions and performed at the Genomic Core Facility at the Hospital for Sick Children in Toronto.

Affymetrix GeneChip data analysis and statistical comparison

Scanned raw data were processed using Affymetrix GeneChip Operating software (GCOS) version 1.4. The intensity value for each probe set was defined at the 75th percentile. To test the integrity of the starting RNA, the ratio of signal intensity of the 3' probe set to that of the 5' probe set for the housekeeping genes (alpha-actin and glyceraldehyde-3-phosphate dehydrogenase) was determined. To monitor the expression of chosen genes in two regions (ischemic versus nonischemic), data obtained from GCOS absolute analyses of all the individual arrays were analyzed and clustered using GeneSpring software 7.0 (Agilent Technologies, USA; www.agilent.com). Differentially expressed genes were clustered according to a hierarchical clustering algorithm by using an average linkage method in GeneSpring. Comparisons were made using Welch's approximate *t* test (which does not assume equality of variances) and ANOVA.

Tissue sections and immunohistochemistry

One-half of each biopsy specimen was fixed in 4% paraformaldehyde in phosphate-buffered saline (pH 7.2) at 4°C and embedded in paraffin. Samples were sectioned to a thickness of 5 μm and mounted on slides; some sections were stained with hematoxylin and eosin. The avidin-biotin complex (ABC) immunoperoxidase method was used for immunohistochemistry (14). Slides were dewaxed and incubated with either rabbit antihuman *Ang-1*, *Ang-2* or VEGF (polyclonal, 1:500 dilution; all from Alpha Diagnostic International, USA) overnight at 4°C . After several washes in phosphate-buffered saline, tissue sections were subsequently incubated with biotinylated anti-rabbit secondary antibody. The ABC Immunoperoxidase Kit (Vector Laboratories, USA) was used according to the manufacturer's instructions with di-aminobenzidine tetrahydrochloride as the colour chromagen. Harris

TABLE 2
Baseline characteristics of the patients

	SA (n=8)	ACS (n=12)
Age, years, mean $\pm$ SD	66 $\pm$ 11	61 $\pm$ 11
Male sex	62	66
Previous myocardial infarction	88	66
Previous percutaneous coronary intervention or coronary artery bypass graft surgery	25	16
Diabetes	62	33
Hypertension	85	92
Hyperlipidemia	85	83
Smoking	72	50
Peak troponin, ng/mL	<0.1	25 $\pm$ 19
Left ventricular ejection fraction, mean $\pm$ SD	38 $\pm$ 12	45 $\pm$ 13

Data presented as % unless otherwise indicated. ACS Acute coronary syndromes; SA Stable angina

hematoxylin was applied as the nuclear counterstain before dehydration and clearing in xylene. Images were captured with a Nikon Eclipse E800 microscope (Nikon Corporation Instruments Company, Japan), using Nikon's ACT-1 digital software. Control slides were incubated with isotype identical rabbit immunoglobulin G instead of primary antibody or by eliminating primary antibody in the process.

RESULTS

Patient population

The baseline characteristics of the patients are summarized in Table 2. Of the 20 patients studied, eight had stable angina and 12 had ACS. Patients with stable angina tended to be older than those with ACS, with a greater incidence of previous MI, coronary revascularization, diabetes and smoking.

Expression of vascular growth factors in ischemic myocardium

Paired ischemic and nonischemic samples from 17 patients (eight stable angina and nine ACS patients) were initially screened for differential changes in gene expression using a panel of reference hypoxia-inducible genes (*HIF1 α* , *VEGF*, *eNOS*, *Flt1* and *Flk1*). The presence of tissue ischemia was validated by increased expression of these genes (1.5- to 15-fold, all $P < 0.05$, ischemic relative to nonischemic samples). None of the patients with stable angina exhibited significant differences in gene expression in samples chosen from within the region targeted for revascularization compared with a control region (data not shown). In contrast, three of nine patients with ACS displayed significant differential changes in gene expression indicative of myocardial ischemia (Figure 1). In these patients, there was markedly increased expression of Ang-2 and, to a lesser extent, Ang-1, as well as the angiopoietin receptors Tie-1 and Tie-2 (all $P < 0.05$). Moreover, expression of the angiopoietins (Ang-1 and Ang-2) was quantitatively greater than that observed for VEGF and its receptors, Flk1 and Flt1. Significant upregulation of the novel VEGF family member, PlGF, was also observed in these samples. Interestingly, one ACS patient showed elevations in Ang-2 and Ang-1 expression, but not for most of the other genes examined. All mRNA transcripts assayed were detectable in nonischemic myocardium to varying degrees (data not shown).

Localization of Ang-1, Ang-2 and VEGF proteins in ischemic myocardium

Immunostaining for Ang-1, Ang-2 and VEGF proteins demonstrated expression in ischemic and, to a lesser extent, nonischemic myocardium, with tissue localization primarily to ECs lining blood vessels (Figure 2). Expression of Ang-1, Ang-2 and VEGF was also observed to a lesser degree in cardiomyocytes and other cell types. Of note, myocardial expression of Ang-1, Ang-2 and VEGF proteins was greatest in the peri-infarct border zone. Furthermore, multiple areas of

Figure 1 Upregulation of the angiopoietin and vascular endothelial growth factor systems in acute coronary syndromes. Quantitative real-time polymerase chain reaction analysis of left ventricular biopsy specimens from acute coronary syndrome patients undergoing urgent surgical revascularization. Gene expression in ischemic samples was compared with nonischemic samples that were arbitrarily assigned a value of 1 unit. Individual patients with significant differential gene expression ($n=3$, $P < 0.05$) are represented by black circles; those without significant changes in gene expression ($n=6$) are represented by open circles. Gene expression analysis was conducted using the relative expression software tool (13). Total messenger RNA expression was normalized to beta-actin. vs Versus

neovascularization were observed within the infarct zone itself, with robust VEGF expression in capillary ECs (Figure 2).

Microarray analysis

Paired intraoperative myocardial biopsies were obtained from three additional ACS patients and subjected to microarray analysis. Patient A was a 74-year-old woman with hyperlipidemia and a family history of coronary artery disease who presented with a posterolateral ST elevation MI and was successfully reperfused with thrombolysis and subsequently experienced post-MI angina. Patient B was a 72-year-old man with coronary artery disease and previous inferior MI, type 2 diabetes, hyperlipidemia and hypertension who presented with an anterolateral non-ST elevation MI. Patient C was a 54-year-old man with hypertension and hyperlipidemia who presented with an inferior STEMI and was successfully reperfused with thrombolysis. All patients received standard medical therapy for ACS, including acetylsalicylic acid, heparin/low molecular weight heparin, beta-blockers, angiotensin-converting enzyme inhibitors and statins.

Data filtering ($P < 0.05$) and hierarchical clustering for the three paired patient samples identified significant upregulation (more than 1.5-fold) in the expression of 114 genes, with significant downregulation (more than 1.5-fold) in the expression of 126 genes (Figure 3). There was remarkable consistency in the expression of individual genes between ischemic and nonischemic regions among the patients (Figure 4).

Consistent with the real-time PCR data, upregulation of Ang-2 was observed to be among the top 10 differentially expressed genes in ischemic myocardium (Table 3). Another important regulator of angiogenesis, caveolin-2, was also among the most highly expressed genes detected by microarray analysis. Conversely, platelet-derived growth factor receptor-beta (PDGFR- β) was found to be downregulated in ischemic myocardium. Upregulation of numerous transcription factors involved in regulating angiogenic gene expression (EHR, FOXF1, NF-YA, nuclear receptor subfamily 2 and WNT9A) was also observed.

A number of genes involved in immune/inflammatory responses also exhibited increased expression in ischemic myocardium, including CCL5/RANTES, interleukin-7 (IL-7) receptor, TNFRSF9/CD137, FLN29 and NOX1 (Table 3). Conversely, there was downregulation of SATB1 – a master transcriptional repressor expressed mainly in T cells that controls expression of multiple genes encoding cytokines, chemokines and apoptosis factors (15). Similarly, decreased expression of Kruppel-like factor 6 (KLF6), a transcription factor expressed by ECs that activates transforming growth factor-beta (TGF- β) signalling in response to vascular injury, was observed (16).

Figure 2) Angiopoietin (Ang)-1, Ang-2 and vascular endothelial growth factor (VEGF) protein localization and neoangiogenesis in ischemic myocardium. Paired photomicrographs showing expression of Ang-1 (A,B), Ang-2 (C,D) and VEGF (E,F) in nonischemic (NI) and ischemic (I) myocardium, respectively. All three proteins localized to endothelial cells lining blood vessels (arrows), with lower expression in cardiomyocytes and the extracellular matrix. Expression of Ang-1, Ang-2 and VEGF was markedly increased in the peri-infarct zone. Multiple areas of neoangiogenesis (arrows) were seen in the infarct zone, with robust VEGF expression in capillary endothelial cells (F,G, inset). Controls incubated without primary antibodies did not show significant staining. Magnifications $\times 20$, inset $\times 40$

Figure 3) Hierarchical clustering of differentially expressed genes in ischemic myocardium. Data filtering and two-way hierarchical cluster analysis identified 240 genes that were differentially expressed in ischemic myocardium. Each ischemic sample was normalized to a nonischemic sample from the same patient. Gene expression levels are depicted as colour variation from red (high expression) to blue (low expression). The colour in each cell of the figure displays the level of expression for each gene (rows) in the ischemic myocardium of each individual subject (column)

A number of genes involved in cardiac remodelling and contractility were found to exhibit differential gene expression. B-type natriuretic peptide (BNP) demonstrated increased expression in ischemic myocardium. Upregulation of PRKCBP1, a receptor for activated C-kinase (RACK) protein that has been shown to bind to activated protein kinase C- β -1, was also observed (17). The G protein-related signalling molecules

Figure 4) Differential gene expression in ischemic myocardium. A total of 240 genes demonstrated differential gene expression. Significant upregulation (more than 1.5-fold, $P < 0.05$; red lines) was observed in the expression of 114 genes, with significant downregulation (more than 1.5-fold, $P < 0.05$; blue lines) in 126 genes. Each ischemic sample was normalized to a nonischemic sample from the same patient

regulator of G-protein signalling 3 and GNAS also exhibited increased expression. As expected, genes involved in post-MI scar formation and fibrosis were also found to be upregulated – including types 1 and 3 collagen. Conversely, expression of MEF2A and GATA4 transcription factors, and the cardiac contractile proteins, myosin light polypeptide 4 and myosin heavy polypeptide 6, was found to be decreased (Table 3).

DISCUSSION

The present study was the first to assess the potential importance of the angiopoietin system in myocardial tissue from patients with cardiac

ischemia. We now show that Ang-2 is highly expressed in ischemic myocardium, to a greater extent than other classical angiogenic factors. Moreover, by unbiased microarray gene profiling, only *Ang-2* emerged among the top 10 differentially upregulated genes, supporting a critical role for this Tie-2 antagonist/partial agonist in mediating the angiogenic response to severe ischemia in the human heart.

Our study is also the first to report myocardial gene expression profiles in patients with ACS. We observed differential expression of a total of 240 genes, with significant upregulation noted in 114 genes and downregulation observed in 126 genes. As expected post-MI, a broad spectrum of gene activation was observed including genes involved in immune/inflammatory responses, cellular proliferation and differentiation, cardiac remodelling, metabolism and apoptosis. Despite the small number of patients assayed, there was remarkable uniformity of differential gene expression (Figures 3 and 4), which is not usually seen in microarray profiling studies comparing samples from different patients or groups. This is likely due to the fact that we compared myocardial samples from the same patient, rather than applying the usual method of comparing myocardial gene expression in samples from different individuals – thus, minimizing the inevitable variability due to differences in genetic background, medications and disease-specific factors, among other confounding factors.

In the initial validation series, we found that only approximately 30% of paired biopsy specimens exhibited differential expression of hypoxia-inducible genes. This likely resulted from the challenges in identifying discrete regions of ischemic and normal myocardium in the clinical context of cardiac revascularization surgery and, thus, inadvertently obtaining paired ischemic or nonischemic samples, despite the great care that was taken both before surgery and intraoperatively to accurately select areas for biopsy.

The angiopoietin system plays a key role in the stabilization and maturation of newly formed vessels (1). Targeted disruption of *Tie-2* or *Ang-1* results in embryonic lethality, occurring at a slightly later stage than that seen in VEGFA knockout animals (18). However, in the postnatal vasculature, Ang-1 appears to play a predominant role in the maintenance of endothelial homeostasis and the prevention of vascular inflammation through the activation of Tie-2 (19). In contrast, Ang-2 is believed to act as an antagonist of Tie-2, releasing the vascular endothelium from tonic inhibitory influence of Ang-1, and facilitating EC activation in response to VEGF and other classical angiogenic factors. Increased Ang-2 expression in biopsies from human ischemic myocardium, localized mainly to ECs lining blood vessels in the peri-infarct border zone, is consistent with the previous demonstration of Ang-2 expression in the leading edge of tumour neovessel formation (20).

However, recent studies have demonstrated that the activity of Ang-2 is more complex than previously believed, and that under certain conditions, particularly during prolonged interaction with the Tie-2 receptor, Ang-2 exhibits partial or even full agonist activity at Tie-2. This time-dependent modulation of Ang-2 activity may be important in the regulation of neovessel formation because one ligand can mediate both the initial inhibition of Tie-2 activity necessary for the efficient early angiogenic response, and the later maturation and stabilization of the neovasculature (1). Indeed, marked upregulation of Ang-2 in the peri-infarct zone – which persisted throughout the period of neovascularization – was demonstrated in a rat MI model (6). In the present study, although Ang-1 expression was reduced, the Tie-2 receptor showed robust activation at three days post-MI, consistent with an agonist role of Ang-2 in this model. However, in human ischemic myocardium, this reciprocal regulation of the major Tie-2 ligands was not seen, and Ang-1 was also found to be elevated – although to a lesser degree. This discrepancy may be due to the possibility that, unlike in experimental models, human tissue is more complex, with both spatial and temporal heterogeneity in ischemia. Nonetheless, the elevation in Ang-2 expression was consistently greater and only Ang-2 was found to be differentially upregulated by microarray gene profiling.

Caveolin-2, another important regulator of angiogenesis (21,22), was also among the most highly expressed genes detected in our analysis.

TABLE 3
Differentially expressed genes in ischemic human myocardium

Upregulated (≥2.0-fold)		Downregulated (≥2.0-fold)	
Ets homologous factor	+3.1	Myosin, light polypeptide 4	–3.2
Protein kinase C binding protein 1	+3.1	SATB1	–2.8
Caveolin-2	+2.9	GCC1	–2.8
Chemokine (C-C motif) ligand 5/RANTES	+2.8	Liver-specific bHLH-Zip transcription factor	–2.7
NADPH oxidase 1	+2.8	Protocadherin 1	–2.5
PSMA7 proteasome subunit, alpha type	+2.8	Myosin, heavy polypeptide 6	–2.4
Methylthioadenosine phosphorylase	+2.5	Kruppel-like factor 6	–2.2
Coagulation factor II (thrombin) receptor-like 2	+2.5	Skeletal embryonic myosin light chain 1	–2.2
Ang-2	+2.4	Transcription factor 8	–2.2
B-type natriuretic peptide	+2.2	PDGF receptor beta	–2.2
Regulator of G-protein signalling 3	+2.2	Ropporin, rhophilin associated protein 1	–2.1
GNAS complex locus	+2.1	Myocyte enhancer factor 2A	–2.1
Interleukin 7 receptor	+2.1	GATA binding protein 4	–2.1
Stearoyl-CoA desaturase	+2.1	RAFTLIN	–2.1
FLN29	+2.0	Follistatin	–2.0

Caveolins coordinate receptor-effector interactions within caveolae, and have been shown to be necessary for postischemic neovascularization mediated by VEGF-NO signalling (21). Moreover, Tie-2 has been shown to be localized in EC caveolae, suggesting a role for caveolae in angiopoietin-induced neovascularization (23). Overall, these data support a model whereby the coordinated action of angiopoietins, VEGF, caveolins and other angiogenic factors result in effective neovascularization in patients with acute coronary syndromes.

We also observed enhanced expression of PlGF and its receptor Flt1 in ischemic myocardium. PlGF is a potent mitogen that has been demonstrated to induce both angiogenesis and arteriogenesis, thereby stimulating capillary and collateral blood vessel formation in ischemic tissue (24). Recently, PlGF plasma levels were reported to be elevated in patients with myocardial infarction and, unlike VEGF, were positively correlated with chronic improvement in left ventricular ejection fraction (25).

The early inflammatory/proliferative phase postinfarction is characterized by increased chemokine expression and infiltration of neutrophils and monocytes/macrophages, along with intense neovascularization. Subsequently, blood vessel maturation and infarct healing occurs during the fibrotic phase, leading to the formation of a stable myocardial scar. Increased PDGF- β expression has been demonstrated in experimental models of myocardial infarction during the fibrotic phase of infarct healing (26). PDGF- β signalling promotes blood vessel maturation by enhancing pericyte recruitment to neovessels. PDGF- β expression is regulated by TGF- β , a key factor in postinfarct remodelling (27). TGF- β , in turn, is regulated by KLF6, a novel transcription factor that is induced by vascular injury (16). TGF- β has been demonstrated to suppress the expression of inflammatory mediators, leading to resolution of the inflammatory infiltrate and transition to the fibrotic phase of infarct healing (27). In the present study, we observed significant downregulation of PDGFR- β and KLF6 expression, consistent with the fact that our biopsy samples were obtained early after ischemia/infarction during the inflammatory phase, when chemokine expression and angiopoietin/VEGF-mediated neovascularization is at its highest.

Atherosclerosis is an inflammatory disease, and the activity of T cells and monocytes/macrophages within coronary plaques has been correlated with the onset of acute coronary syndromes (28). In the present study, we observed increased expression of CCL5/RANTES, IL-7 receptor and NOX1. CCL5/RANTES is a pivotal chemokine that mediates peripheral

monocyte recruitment into sites of arterial injury, and has been implicated in the pathogenesis of plaque progression and rupture (29). CCL5/RANTES is released by activated platelets, and elevated plasma levels of CCL5/RANTES have been detected in patients with ACS (30). Our finding of increased CCL5/RANTES expression may reflect increased local myocardial expression downstream from the culprit plaque by activated platelets or, alternatively, by endothelial or smooth muscle cells. IL-7 is a key mediator of T cell activation and, recently, has been shown to be elevated in patients with ACS (31). IL-7 is released by activated platelets and causes monocytes to release proatherogenic chemokines including IL-8, MCP-1, MIP-1a and IP-10. Conversely, we observed significant downregulation of SATB1, a master transcriptional repressor of cytokine and chemokine expression (15), presumably further potentiating the inflammatory response early postinfarction.

NOX proteins have been suggested to play a role in atherosclerotic plaque progression through the production of reactive oxygen species (ROS) (32). Interestingly, MCP-1 has been shown to induce production of ROS from the peripheral monocytes of patients with unstable angina (33), prompting the suggestion that chemokine-induced ROS production by monocytes within vulnerable plaques causes apoptosis, thereby enhancing metalloproteinase activity, and leading to plaque

rupture and ACS. Although the exact roles of CCL5/RANTES, IL-7 receptor and NOX1 in ACS remain to be clarified, our observations strongly suggest a critical role for immune and inflammatory mediators in triggering both ACS, as well as the subsequent myocardial response to ischemia, likely contributing to tissue injury and scar formation.

CONCLUSION

The present report of microarray analysis in patients with acute coronary syndromes identifies *Ang-2* as a potentially important gene in the angiogenic response of the human heart. Our results also highlight the role of immune/inflammatory molecules in the pathogenesis of ACS. Common gene expression patterns in ACS may provide opportunities for targeted pharmacological and cellular intervention.

DISCLOSURES: The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

FUNDING SOURCES: Supported by the Canadian Institutes of Health Research (MOP – 160148) and Physicians' Services Incorporated Foundation (grant R02-26).

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