Laminar Shear Stress Differentially Modulates Gene Expression of p120 Catenin, Kaiso Transcription Factor, and Vascular Endothelial Cadherin in Human Coronary Artery Endothelial Cells*

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We demonstrated previously that laminar shear stress (LSS) enhances human coronary artery endothelial cell (HCAEC) wound closure via a vascular endothelial cadherin (VE-cadherin)-dependent mechanism. VE-cadherin can interact with p120 catenin (p120ctn) to mediate cell locomotion and proliferation. In this study, we hypothesized that p120ctn and an interacting protein, Kaiso, a transcriptional factor with which p120ctn may interact, would be expressed differentially at the wound border and away from the wound border in HCAEC exposed to LSS. One of the major goals in this study was to assess the differential gene expression of p120ctn, Kaiso, and VE-cadherin in HCAEC at specific locations along the wound border to further our understanding of the molecular mechanisms involved in wound closure. We combined the technique of laser capture microdissection with quantitative real time PCR to compare p120ctn, Kaiso, and VE-cadherin mRNA expression in HCAEC at and away from the wound border under LSS. Total RNA was isolated from 200–1,000 laser-captured HCAEC and reverse transcribed into cDNA. Detection of p120ctn, Kaiso, and VE-cadherin mRNA was carried out using quantitative real time PCR. Normalization of cDNA templates was achieved by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) quantification. Quantitative real time PCR analysis revealed p120ctn/GAPDH ratios, Kaiso: GAPDH ratios, and VE-cadherin/GAPDH ratios, relative to static control for each set, of 0.99–4.18 (mean ± S.E., 1.94 ± 0.404), 1.0–5.24 (2.11 ± 0.51), and 0.99–4.42 (1.09 ± 0.09) after 3 h of LSS, respectively. With these techniques, we found that p120ctn and Kaiso transcripts were increased in laser-captured HCAEC at the wound border compared with HCAEC away from the wound border. In addition, differential expression of p120ctn and Kaiso mRNA was observed in HCAEC depending on how LSS was applied in relation to the wounding process. These techniques may have wide applicability for studying wound healing because gene expression of key adhesion molecules in HCAEC may now be determined from select regions of the endothelial wound border.

Adherens junctions between endothelial cells are important for maintaining vascular integrity. Vascular endothelial cadherin (VE-cadherin) expressed by endothelial cells contains cytoplasmic sequences that anchor the adherens junctions to p120 catenin (p120ctn) and β-catenin adhesion molecules important for cell migration and proliferation (1, 2). Unlike β-catenin that directly links VE-cadherin to the actin cytoskeleton via α-catenin, p120ctn regulation of the actin cytoskeleton is thought to occur via the Rho family of GTPases (3). The importance of p120ctn in endothelial cell motility, adhesion, and migration (4) has been highlighted recently by the additional discovery of Kaiso, a protein that interacts with p120ctn and which is speculated to have a possible nuclear signaling function (4, 5).

Vascular endothelial cells lining our blood vessels are constantly exposed to the hemodynamic forces of blood flow which produce a tangential force, a shear stress, upon the endothelial cell surfaces. Several laboratories including our own (6–11) have demonstrated that shear stress can modulate the expression of genes critical to vascular integrity (12–14). However, the effects of laminar shear stress (LSS) on specific cells along a wound border and their gene expression of key adhesion molecules have not been examined. We demonstrated previously that LSS increases VE-cadherin protein expression, immunolocalization, and translocation during the process of wound closure (9). However, we relied on immunohistochemical techniques to detect changes in expression along a wound border, and protein expression by Western blot was reflective of the entire monolayer and not of specific cells along the wound border. Through adaptation and refinement of the techniques of laser capture microdissection (LCM) and real time PCR, we are now able to determine specifically expression of a given adhesion molecule mRNA at the wound border. In the current report we hypothesized that the gene expression of VE-cadherin, p120ctn, and Kaiso, a transcription factor putatively for p120ctn, would be regulated differentially by LSS and dependent on the specific location of the cells along the wound border and away from the wound border.

Most techniques for assessing gene expression such as Northern blot analysis, RNase protection assay, and in situ hybridization cannot produce quantitative, cell-specific gene expression data. However, with microdissection methods one can selectively obtain pure cell populations from tissue sections

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for RNA analysis (15–17). The development of laser capture microdissection (LCM) and high-through-put mRNA analysis platforms may therefore provide a unique and powerful opportunity to assess directly the molecular and genetic events that are associated with the initiation and progression of wound closure in human coronary artery endothelial cells (HCAEC) subjected to LSS. Because LCM techniques have been optimized for tissue sections and not for cells grown on a glass slide, this is the first report to demonstrate the feasibility of LCM to capture and study the gene expression of specific cells in a wounded monolayer model of the coronary artery endothelium.

In this study we also report the obstacles faced in optimally applying LCM to gene expression analysis of p120ctn, Kaiso, and VE-cadherin in cultured HCAEC. The development of LCM has challenged the sensitivity of any chosen molecular assay because the amount of material obtained by LCM is limited. We present here an effective method for mRNA quantification for gene expression profiling from as few as 200 cells obtained by LCM followed by quantitative real time PCR analysis. We did not experience the RNA degradation that may occur during LCM procedures. By applying the techniques of LCM and real time PCR, we are now better positioned to study adhesion molecule gene expression in endothelial cells involved in the process of human coronary artery wound healing.

EXPERIMENTAL PROCEDURES

Cell Culture—HCAEC were purchased from Clonetics, Inc. (San Diego) and cultured in Endothelial Growth Medium-2 (EGM-2, Clonetics, Inc.) containing 10% fetal bovine serum. HCAEC of six or seven passages were used in the experiments, and used at 80–100% confluence by the time of the experiment.

Application of Shear Stress—HCAEC monolayers were placed in a parallel plate flow chamber (CytoShear) and subjected to two different protocols as described previously (9–11, 18). In the first protocol, HCAEC were preconditioned by applying a LSS of 20 dynes/cm² for 6 h to achieve alignment, wounded with a metal spatula, and finally reseeded at 20 dynes/cm² for 1 or 3 h or left in static maintenance. In the second protocol, HCAEC were not preconditioned but instead immediately wounded and subjected to a LSS of 20 dynes/cm² for 1 or 3 h or left in static maintenance. All HCAEC monolayers were maintained at 37 °C and 5% CO₂.

LCM and Processing of HCAEC—Cells were washed with PBS and dehydrated in a graded series of 75, 95, and 100% ethanol for 1 min each. Finally, cells were submersed for 3 min in pure xylene, air dried dehydrated in a graded series of 75, 95, and 100% ethanol for 1 min at 37 °C, and then are associated with the initiation and progression of wound healing. Platforms may therefore provide a unique and powerful opportunity for RNA analysis (15–17).

Optimization of the Reverse Transcription (RT) Reaction—RT was carried out in a one-step 20 μl reaction with Superscript II RNAse H⁻ (Invitrogen) or with MuLV reverse transcriptase (Roche Applied Science) using an oligo(dT) primer and 10 ng of RNA according to the manufacturer’s protocol.

Real Time PCR Optimization—Real time PCR was performed either directly from RNA samples (one-step) or from reverse transcribed cDNA samples (two-step) for relative quantification of mRNA levels for the genes of interest and to determine which method, one-step or two-step, was of superior reproducibility. Quantitative real time PCR was performed using TaqMan® universal PCR master mix. Primers and TaqMan® probes were designed using PrimerExpress 1.0 software (PerkinElmer Life Sciences) and detailed in Table I. Although p120ctn is expressed as multiple isoforms that share the central armadillo repeat domain but have divergent N and C termini, in the present study primers were designed to flank the conserved region shared by all isoforms of p120ctn and not targeted for a particular isoform (Table I). The cDNA was diluted to 5-fold, and a 1 μl was used as a template in each PCR with PCR TaqMan® universal master mix containing 1× PCR buffer and the appropriate concentrations of gene-specific primers, TaqMan® probe (Table I), 5 mM MgCl₂, 0.025 unit AmpliTaq Gold, and 0.2 mM dNTPs combined in a total volume of 25 μl. A negative control lacking RT enzyme was also included in each assay. For the one-step PCR method, 1 μl of 5-fold diluted RNA and MuLV reverse transcriptase were included in the reaction mixture. The PCR cycling for one-step PCR was performed at 42 °C for 30 min (reverse transcriptase reaction) followed by 95 °C for 10 min (denaturation) and then 95 °C for 15 s and 60 °C for 1 min for a total of 40 cycles. The same cycling parameters were followed for two-step PCR with the exception of the RT reaction. The cycle threshold (Ct) values corresponding to the PCR cycle number at which fluorescence emission in real time reaches a threshold above the base-line emission were determined. The Ct value assigned to a particular well thus reflects the point during the reaction at which a sufficient number of amplicons have accumulated, in that well, to be at a statistically significant level above base line.

Effect of LCM Processing on RNA Quality—Two sets of replicates of 100 and 1,000 HCAEC grown on glass slides were used in this study. One set of slides was simply washed with PBS, and the other set was processed for LCM as described previously. The total RNA isolated from both sets of samples was reverse transcribed with Superscript II RNAse H⁻ according to the supplier’s instructions. The cDNA was diluted 10
times, and 4 μl of template was used in 25-μl PCRs for real time PCR and detection of GAPDH-specific amplification.

**RNA Yield and Laser Pulses**—RNA was extracted and reverse transcribed from LCM samples containing HCAEC captured with 200, 400, 800, or 1,000 laser pulses. 1 μl of cDNA from each sample was used in a 25-μl PCR to determine the Ct values of GAPDH gene by real time PCR.

**Quantitative Real Time PCR**—Real time PCR was performed for amplification of p120ctn, Kaiso, and VE-cadherin mRNA using two-step PCR. In each assay, PCRs were performed in duplicate for the standards and unknown samples to determine the relative quantities of GAPDH, p120ctn, Kaiso, and VE-cadherin amplicons. To compare expression patterns in HCAEC, mRNA template concentrations for GAPDH and the target genes were calculated using the standard curve method. Standard curves were constructed using 5-fold dilutions of HCAEC cDNA (1, 0.2, 0.04, 0.08, and 0.016 ng). The mRNA quantity of each amplicon was calculated for each standard and experimental sample. After normalization for GAPDH, target genes were expressed relative to the static control away from the wound border. End products of the PCR were run on a 2% agarose gel for confirmation of the correct amplicon size and identification of a single PCR band.

**Immunofluorescence**—HCAEC monolayers subjected to experimental protocols were rinsed in PBS containing calcium and magnesium and fixed in ice-cold methanol for 10 min. Cells were blocked in 10% normal goat serum in PBS, incubated in primary antibodies (rabbit anti-p120ctn, Santa Cruz, Santa Cruz, CA; or mouse anti-Kaiso, Zymed Laboratories Inc., San Francisco) diluted 1:500 in PBS, and visualized with species appropriate secondary IgG conjugated with Rhodamine Red (Molecular Probes, Eugene, OR) diluted 1:250 in PBS. Coverslips were placed over the slides with Aqua Poly Mount, and unsaturated images at and away from the wound edge were acquired using a Zeiss Axioplan microscope equipped with a 40× oil objective. Images were analyzed for relative fluorescence intensity using Metafluor software version 4.5r (Universal Imaging, West Chester, PA). Confocal images were color separated, and three measurement lines drawn across regions of the image spanning six cells and five cell junctions. Pixel intensity was measured over a scanning width of eight pixels along each line, and intensities of the three lines were averaged for each experimental condition in three separate experiments at the time point of 3 h. Pixel intensities were normalized for average background on each slide. For both p120ctn and Kaiso, average intensities were normalized to and expressed as a percentage of the static control for that experimental condition.

**Data Analysis**—Samples of two different reactions from three independent experiments were grouped for statistical analysis. The efficiencies of PCRs using various primer sets were similar, and standard curve methods were performed to quantify relative amounts of transcripts as means ± S.E. Student's t test was used to test for differences of means between the groups. All probability (p) values were based on two-tailed t tests, and p ≤ 0.05 was considered to be significant.

**RESULTS**

**LCM of HCAEC**—Cell morphology with respect to cytoplasmic and nuclear details was well preserved after dehydration as shown in Fig. 1. HCAEC from the wound border and away from wound edge were captured. As shown In Fig. 1, HCAEC are indicated at the wound border (A), during microdissection (B), after capture (C), and on the cap (D). The arrows in A and C point to some representative cells captured at the wound border. Isolation efficiency was high with all HCAEC targeted by the laser being transferred to the capture disc. For some of the cell preparations, the strength and duration of the laser pulse were increased above the initial settings of 40 mW for 1.0 ms to ensure that cells adhered to the cap. Based on subsequent analysis, the use of longer duration (2 ms) and higher voltage (100 mW) for LCM capture were used and caused no damage to the RNA.

**RNA Quality**—To determine whether RNA isolated from cells via dehydration and LCM was of the same quality as RNA isolated from cells that were not processed by LCM, RNA was isolated from 100 or 1,000 HCAEC by both techniques. The mean Ct values for the 100 and 1,000 PBS-washed (29.30 and 24.30, respectively) and dehydrated cells (28.80 and 23.90, respectively) were found to be similar (Fig. 2). The RNA isolated from LCM samples containing HCAEC captured with 200, 400, 800, and 1,000 laser pulses. As shown in Fig. 3, the Ct values of the cDNAs from LCM samples obtained by real time PCR for GAPDH amplicon plotted against the number of pulses gave a correlation coefficient of 0.92. This demonstrates a linear relationship between the number of pulses and the Ct values (Fig. 3).

**RT Efficiency**—To determine which reverse transcriptase was more efficient in transcribing RNA to cDNA, two popular commercially available enzymes were used in this study. The results obtained with first strand cDNA synthesis by Superscript II RNase H− (Fig. 4, curves 1 and 3) and MuLV (Fig. 4, curves 2 and 4) by real time PCR, based on the Ct values for each cDNA dilution, were almost identical. The differences in efficiency were negligible. Analysis showed similar amplification plots and Ct values for both reverse transcriptases.

**Standard Curve Method for Determining Relative Quantities of Gene Expression**—The standard curves for GAPDH (Fig. 5A), p120ctn, Kaiso, and VE-cadherin amplicons (data not shown) were generated by plotting Ct values versus log ng of cDNA. The slope of the regression line was used to calculate the amount of cDNA, and thus mRNA, in each sample. In Fig. 5B, amplification plots (fluorescence data versus cycle number) of GAPDH indicate the Ct values for each cDNA dilution. The mRNA expression levels of target genes were normalized to GAPDH mRNA.

**Expression Levels of p120ctn, Kaiso, and VE-cadherin mRNA**—In an attempt to understand the role of p120ctn, Kaiso, and VE-cadherin in HCAEC wound closure under LSS, the mRNA expression patterns in HCAEC at and away from the wound border were compared with and without preconditioning prior to wounding and exposure to LSS or static maintenance for 1 h (Fig. 6A) and 3 h (Fig. 6B). The expression levels of p120ctn, Kaiso, and VE-cadherin were normalized to GAPDH mRNA expression and then expressed relative to the static control away from the wound border for that experimental condition. As we have reported previously, preconditioning allows for a more physiologic milieu for HCAEC and a closer approximation to the state of HCAEC that line the coronary vasculature (9–11, 18). The static control for this condition
received 18 h of preconditioning with LSS prior to wounding and reexposure to LSS and differs from the static control of HCAEC that were not preconditioned and exposed to LSS only after the wounding process. RNA obtained from LCM samples (600 laser shots) was enough for analysis of three genes. Ethidium bromide-stained PCR products after real time PCR confirmed specific amplification for the three genes of interest (Fig. 7). As shown in Fig. 6, we selected the static control away from the wound border as the base-line control for each experimental condition. HCAEC in the more physiologic environment afforded by preconditioning demonstrated significantly greater p120\(^{ctn}\) and Kaiso expression in cells at the wound border compared with cells away from the wound border at both time points. At 1 h (Fig. 6A) imposition of LSS after preconditioning produced a more robust and significant increase in p120\(^{ctn}\) expression at the wound border compared with cells away from the wound edge and compared with

**Fig. 2.** Integrity of RNA quality from LCM cells. RNA was isolated from 100 or 1,000 HCAEC (washed with PBS or LCM processed). The isolated RNA was reverse transcribed and subjected to two-step real time PCR using primers and a TaqMan® probe for GAPDH amplicon. Both cDNAs generated almost identical GAPDH Ct values by PCR.

**Fig. 3.** A linear relationship between LCM pulses and RNA yield. Cells were captured using 200, 400, 800, or 1,000 laser pulses from HCAEC wounded monolayers. The isolated RNA was subjected to two-step real time PCR using primers and a TaqMan® probe for GAPDH amplicon. The standard curve of Ct value versus the number of pulses yielded a correlation, R value, of 0.92.

p120\(^{ctn}\) expression at 3 h (Fig. 6B). In preconditioned HCAEC that were subsequently left in static maintenance, p120\(^{ctn}\) expression was increased significantly at the wound border compared with cells away from the wound edge at 1 h but not at 3 h. HCAEC that had not been preconditioned but rather were subjected to the immediate forces of LSS only after wounding had less robust p120\(^{ctn}\) mRNA expression at 1 h compared with 3 h. However, despite the absence of preconditioning there remained a difference in p120\(^{ctn}\) expression in cultured HCAEC at the wound border compared with cells away from the wound edge in this set. Thus, there seems to be an effect of the wounding process on p120\(^{ctn}\) mRNA expression which is significantly augmented by LSS in HCAEC at 1 h after preconditioning and at 3 h without preconditioning. In both cases, p120\(^{ctn}\) mRNA expression was increased significantly by LSS, albeit to different degrees. These data suggest that the physiologic environment of preconditioning is associated with earlier increases in p120\(^{ctn}\) mRNA expression relative to cultured HCAEC lacking this milieu. The mRNA expression of Kaiso was increased in cells at the wound border compared with cells away from the wound edge at both time points but only in preconditioned HCAEC (Fig. 6, A and B). There were no significant differences in VE-cadherin mRNA expression at either time point.

**Immunolocalization**—All immunolabeling was performed 3 h after the wounding process. With respect to protein expression, p120\(^{ctn}\) relative immunofluorescence nearly doubled at the wound border (relative intensity 190% of control) compared with cells away from the wound edge (relative intensity 60% of control) in preconditioned HCAEC and compared with HCAEC left in static maintenance (p = 0.0001, data not shown). As seen in Fig. 8, preconditioned HCAEC that were reexposed to LSS after wounding demonstrated greater immunofluorescence in the cytoplasm of cells at the wound edge (B, arrowheads), and to a lesser but still significant degree at cell-cell junctions, compared with HCAEC away from the wound edge (A). In all cases Kaiso protein expression was significantly greater at the wound edge com-
pared with cells away from the wound border, but preconditioning did not yield greater protein expression compared with cells that had not been preconditioned. In HCAEC that were not preconditioned, Kaiso protein expression was enhanced at the wound edge (D, arrows) compared with cells away from the wound edge (C). These data suggest that Kaiso protein expression is increased at the wound border and may not solely depend on LSS for its protein expression. We recognize that there may be limitations in comparing immunofluorescence intensity from different samples because of the possibility of differences in antibody binding and nonspecific immunofluorescence. However, every attempt was made to process the monolayers under the same conditions and always to group a shear and static monolayer for processing of immunostaining. Another limitation of the protein data is that with the current LCM technology we were not able to perform Western blotting on specific cells at and away from the wound edge. At least 10,000 cells are required for such a determination. Thus, Western blotting cannot yet be practically performed on a few specific cells at the wound border and compared with a few specific cells away from the edge. In a previous study we performed Western blots on the entirety of the wounded HCAEC monolayers and detected a difference in VE-cadherin protein expression with LSS (9). Immunocytochemistry of the wound edge confirmed an increase in VE-cadherin protein expression (9). In this report, confocal microscopy of immunofluorescent sections suggests significant differences in p120ctn and Kaiso expression as detailed above.
p120ctn, Kaiso, and VE-cadherin Gene Expression in HCAEC

DISCUSSION

In the present study we were able to use the technique of LCM in a novel fashion with real time PCR to quantify p120ctn, the transcriptional factor Kaiso, and VE-cadherin mRNA expression in wounded HCAEC exposed to LSS. We found that preconditioning compared with no preconditioning differentially affected the mRNA expression of p120ctn and Kaiso at and away from the wound border. There was a marked increase in Kaiso and p120ctn expression after a 1-h or 3-h exposure to LSS in HCAEC at the wound border. LSS applied in the fashion seen in the vasculature with a period of preconditioning, followed by wounding and reexposure to LSS, resulted in increased Kaiso and p120ctn transcript levels at 1 and 3 h. For p120ctn expression, there was a time-dependent up-regulation of expression by LSS on HCAEC at the wound border which was more robust at 1 h compared with 3 h. This suggests an attenuation of p120ctn expression with prolonged exposure to LSS after preconditioning. However, in HCAEC that were not preconditioned p120ctn expression was more robust at 3 h, suggesting the need for at least 3 h of stimulation by LSS to see significant mRNA induction. Only preconditioned HCAEC showed significantly increased Kaiso mRNA expression at the wound border compared with cells away from the wound edge at 1 and 3 h. Kaiso mRNA expression was not enhanced significantly in HCAEC that were not preconditioned, suggesting the need for prolonged stimulation with LSS to increase mRNA expression. In addition, this finding suggests that studies of Kaiso expression in HCAEC from vascular endothelial cells are more appropriately determined in the physiological setting of preconditioning. We found no significant change in VE-cadherin mRNA expression between HCAEC at and away from the wound border under any condition. These are the first data to implicate a possible role for LSS in the induction of p120ctn and Kaiso transcription factor mRNA expression during wound closure and to demonstrate the differential response in these genes in HCAEC at the wound border compared with cells away from the wound border.

Several technical difficulties had to be overcome to perform LCM on our cell monolayers accurately. Until these data, LCM has been generally applied to the analysis of cells from tissue slices. Cells on a glass slide, however, present a greater challenge for optimal LCM capture given the amount of matrix they deposit and their consequent adhesivity onto the glass slide. The condition of the macromolecules to be analyzed must be preserved, and optimum processing is required for the most stringent and quantitative gene expression analysis (19). We were able to overcome these difficulties by implementation of a rapid dehydration protocol, select LCM laser settings, and the use of the PicoPure RNA isolation kit for optimal extraction of RNA from the captured HCAEC. Generally, quantitative real time PCR is the most common method used for measuring mRNA levels from small numbers of cells. This type of analysis is handicapped, however, by the difficulties associated with isolating RNA from small samples such as fixed cells or LCM cells. Some investigators have used the Agilent 2100 Bioanalyzer to determine the quality of RNA and peaks corresponding to the 18 S and 28 S rRNAs (www.ambion.com/techlib/tn/92/ 922.html). In this study, the quantity and quality of the RNA isolated from LCM samples were determined by real time PCR based on Ct values. The Ct values for the normal and dehydrated cells were found to be equivalent.

In this report, RNA isolation using the PicoPure RNA isolation kit and reverse transcription using Superscript II RNase H– or MuLV reverse transcriptase provided efficient and sensitive methods for cDNA synthesis of small amounts of RNA. Superscript II RNase H– and MuLV enzymes gave similar cDNA yields, and either enzyme could be used in the RT reactions for transcribing the small amounts of RNA. Another technical difficulty in gene expression analysis of cells obtained by LCM is availability of the extremely small amounts of sample. In the present study at least 600 laser pulses were utilized to obtain sufficient RNA for analysis of three genes with appropriate replicates.

The widely distributed one-step real time PCR model was tested but not applied subsequently because individual and slightly different RT conditions would potentially occur in each reaction setup and for each investigated factor. Thus, in this report we used the two-step real time PCR because of increased sensitivity and reproducibility. The requirements for a proper internal control gene were met by normalization of the target gene with the endogenous standard GAPDH to compensate for inter-PCR variations with respect to RNA integrity and sample loading (20). Although housekeeping gene expression has been reported to vary considerably with different treatments, we did not find any up-regulation of GAPDH in HCAEC exposed to LSS (20, 21). Using identical number of cells/tube, mRNA expression of GAPDH in HCAEC did not differ significantly between LCM samples.

Interactions among the different catenin proteins suggest that p120ctn may participate in regulating the function of cadherins and, thereby, other processes influenced by cell-cell adhesion (2). VE-cadherin, expressed in endothelial cells, is involved in morphogenesis, regulation of permeability, and cellular proliferation. The cytoplasmic tails of cadherins contain the juxtamembrane domain that plays a role in the intercellular localization of the protein and also serves to bind p120ctn. p120ctn plays a key role in cell migration as well as cell adhesion and is used by VE-cadherin to mediate cell motility (4). Although p120ctn binds to VE-cadherin at the juxtamembrane...
brane domain, it may translocate to the nucleus in complex with the transcription factor Kaiso for promotion of cellular motility (22). There is a growing body of data to suggest that p120ctn binding to cadherin at the proximal juxtamembrane domain allows it to participate and modulate the function of small G proteins involved in cellular motility (4, 12). It is speculated that the nuclear functions of Kaiso may also be linked directly or indirectly to adhesive events taking place at the plasma membrane and/or involved in actin-associated signaling events contributing to cellular motility. The physiological roles of Kaiso and p120ctn will be more easily addressed following the molecular identification of the Kaiso gene targets (5, 12). In future studies, it will be important to resolve the signaling pathways governing the expression and activity of Kaiso, which in turn are likely to be linked to those influencing p120ctn expression (22, 23).

Several investigators have reported that shear stress modulates pathophysiologically relevant genes, such as intracellular adhesion molecule-1 (7), platelet-derived growth factor-B (24), monocyte chemotactic protein-1 (25), and early growth response-1 (26). In a recent study, it was reported that preconditioning significantly increased the expression of nitric-oxide synthase-3, fibroblast growth factor-2, vascular endothelial growth factor, and insulin-like growth factor-1 but decreased the endothelin-1 (END1) and platelet-derived growth factor-B expression levels compared with the no-flow conditions (27). We were the first group to routinely use a model of preconditioning to simulate the physiological in vivo state of HCAEC (as seen in the vasculature) prior to wounding and re-shearing (9–11, 18). However, because prior to this report we had never tested the hypothesis that p120ctn and Kaiso mRNA expression would differ with the duration and manner in which LSS was applied, we judged it important to include an experimental set that did not receive preconditioning. In a previous study, we demonstrated that VE-cadherin protein expression of wounded HCAEC monolayers was enhanced by LSS, and, from immunohistochemical analysis, that VE-cadherin signal intensity was increased significantly in HCAEC at the wound border under LSS (9). However, immunohistochemical techniques alone were used to detect changes in expression along a wound border, and protein expression by Western blot was reflective of the entire monolayer and not of specific cells along the wound border. In the current study, we found significant differences at the mRNA level for Kaiso and p120ctn but not VE-cadherin in HCAEC at the wound border compared with away from the wound border, and with or without preconditioning. In terms of protein expression, we found that p120ctn mRNA levels were increased significantly in preconditioned HCAEC at the wound border showed significantly increased cytoplasmic, and, to a lesser but still significant degree, increase in junctional intensities compared with cells away from the wound edge. These data suggest a role for enhancement of p120ctn protein expression by LSS and translocation to the cytoplasm in cells at the wound edge. We speculate that such translocation may aid p120ctn interactions with the Rho family of Ras-related small GTPases for cellular locomotion. Kaiso protein expression, on the other hand, was increased in cells at the wound border compared with cells away from the wound edge under all conditions. However, there were no changes in its localization, and its protein expression may be more dependent on the wounding process itself rather than solely by stimulation with LSS. These discrepancies between mRNA and protein expression highlight the potential weakness of quantitating expression at the mRNA level regardless of the elegance of the laser capture microdissection technique. In addition, unlike the case for mRNA, LCM has not yet been refined enough to perform protein determinations in a practical fashion on a few specific cells at the wound border compared with a few specific cells away from the wound edge.

In summary, these are the first data to suggest that it is possible to use LCM to analyze gene expression of select cells from a single monolayer of endothelial cells cultured on a glass coverslip and to demonstrate the feasibility of applying LCM and quantitative real time PCR techniques for determination of mRNA expression from specific regions of the wounded monolayer. Our studies show that p120ctn and Kaiso mRNA expression varied up to 5-fold in HCAEC microdissected from the wound border compared with HCAEC away from the wound border in response to LSS and that there were no significant differences in mRNA expression of VE-cadherin under preconditioning or with immediate exposure to LSS. This suggests that VE-cadherin may mediate its role in wound closure by mRNA stabilization and prolongation of its half-life or by modification of translation (9) and post-translational processes rather than by induction of mRNA as reported previously (8). p120ctn may act essentially to stabilize or elevate VE-cadherin levels. For example, it has been observed by Iretón et al. (28) that in the case of E-cadherin, levels increased substantially in SW48 epithelial cells upon reconstitution of p120ctn because of a doubling of the cadherin half-life. Other potential explanations include that p120ctn may competitively block interactions of VE-cadherin with other factors that promote VE-cadherin recycling or degradation.

The data in this study substantiate our hypothesis that p120ctn and Kaiso mRNA expression is regulated differentially by shear stress during HCAEC wound closure. In addition, these data point to the need for an appropriate physiological milieu, as attained with preconditioning, to detect significant differences in Kaiso mRNA expression at the wound border compared with away from the wound border. Our results implicate a role for Kaiso as a transcriptional factor in HCAEC in a model of wound closure. These findings of cell specific gene expression may further our understanding of molecular events that take place along an endothelial wound and which may be critical for endothelial wound repair in the coronary circulation.

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