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Endothelial monocyte activating polypeptide-II induced gene expression changes in endothelial cells

Anita T. Tandle[a], Chiara Mazzanti[a], H. Richard Alexander[a], David D. Roberts[b], Steven K. Libutti[a,*]

[a] *Surgery Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA*
[b] *Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA*

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Abstract

In the current study we used microarray (MA) analysis to examine gene expression changes in human umbilical vein endothelial cells (HUVEC) exposed to the tumor-derived cytokine, endothelial monocyte-activating polypeptide-II (EMAP-II). HUVEC treated with EMAP-II for 0.5, 1, 2, 4 and 8 h, were analyzed using 10K cDNA arrays. Our results demonstrated that changes in gene expression of <0.5 and >2 fold were seen for 69 genes and the majority of gene changes occurred early. Validation of MA analysis for 10 genes by real time RT-PCR, demonstrated the gene changes to be consistent and specific to HUVEC when compared to human fibroblasts treated with EMAP-II. Among these genes, downregulated in ovarian cancer 1 (DOC1) gene was studied further because of its possible role in EMAP-II induced cytoskeletal remodeling. DOC1 expression was silenced using small interfering RNA. SiRNA to DOC1 completely abolished EMAP-II stimulated gene expression of DOC1. Silencing of DOC1 gene expression reversed the modulatory effect of EMAP-II on 4 other genes, suggesting that DOC1 might play a role in mediating some of the effects of EMAP-II on endothelial cells.
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Keywords: DOC1; EMAP-II; Endothelial cells; Microarray analysis; SiRNA

1. Introduction

The proinflammatory cytokine endothelial monocyte activating polypeptide-II (EMAP-II) was first detected in the supernatants of murine tumor cells by virtue of its ability to stimulate endothelial-dependent coagulation in vitro [1]. The mature 23 kDa form of EMAP-II, for which the biological activities have been described, is synthesized as a precursor protein lacking a conventional secretion signal peptide [2]. It has been shown that caspase-7 cleaves and releases mature EMAP-II in vitro from proEMAP-II [3]. Hypoxia is also a potent inducer of release of biologically active EMAP-II [4].

Purified EMAP-II has pleiotropic effects on endothelial cells (ECs), monocytes, and neutrophils. In addition to the induction of tissue factor-dependent coagulation on ECs and monocytes, EMAP-II up-regulates endothelial E- and P-selectin expression and stimulates the release of von Willebrand factor. It acts as a chemoattractant for neutrophils and monocytes and induces release of myeloperoxidase activity from neutrophils [2]. It inhibits EC proliferation by binding to α-adenosine triphosphate synthase [5]. EMAP-II can also inhibit neovascularization, in both matrigel and corneal angiogenesis models [6,7].

The effects of EMAP-II on tumor growth are complex. EMAP-II abrogates tumor growth by induction of EC

* Corresponding author at Surgery Branch, Center for Cancer Research, National Cancer Institute, 10 Center Drive, Building 10, Room 4W-5940, Bethesda, MD 20892-1201, USA. Tel.: +1 301 496 5049; fax: +1 301 402 1788.
E-mail address: libuttis@mail.nih.gov (S.K. Libutti).

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apoptosis in vivo [3,6–8]. It also sensitizes tumors to the effect of tumor necrosis factor (TNF)-α [9,10]. It has been shown that EMAP-II upregulates TNF-receptor 1 expression by ECs both in vitro and in vivo [7]. The induction of TNF receptor 1 expression may be the mechanism by which EMAP-II sensitizes tumor endothelium to the effects of TNF leading to hemorrhagic necrosis.

The regulation of gene expression is a fundamental step in cellular physiology. With previous molecular techniques, regulation of gene expression could only be studied on a small number of genes at a time. Newly developed molecular genetic and computational technology has enabled us to analyze differential gene expression profiles of thousands of genes collectively in response to a single stimulus. DNA microarray (MA) technology represents a powerful tool for rapid, comprehensive, and quantitative analysis of gene expression profiles of normal/disease states and developmental processes [11–13]. This approach is ideally suited for studying the pattern of gene expression in ECs induced by various experimental conditions [14].

EMAP-II is a multifunctional protein, and there has been intense study of its effects on various cell types in vitro and in vivo [2,6,7,9,10]. However, the molecular mechanisms behind its action are not known. In the current study, we have used cDNA MA to examine changes in the expression profile of various genes in human umbilical vein endothelial cells (HUVEC) in response to EMAP-II treatment, in order to gain a better understanding of the specific pathways involved in the activity of EMAP-II on ECs.

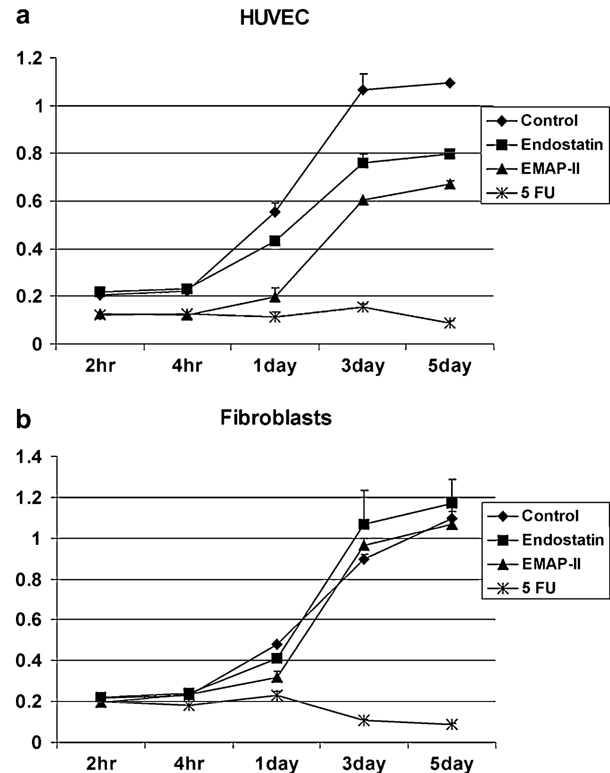

Fig. 1. Cell proliferation assay. HUVEC (a) or human fibroblasts (b) (2000/well) were plated and treated with carrier control, 5-FU (50 μg/ml) and Endostatin (10 μg/ml) as a positive control and 10 μg/ml EMAP-II for 2 h, 4 h, 1 day, 3 days and 5 days. Inhibition of cell proliferation was tested using WST reagent and viable cell number measured at 440 nm. The line graphs were plotted using treatment time on the X axis against the OD readings on the Y axis. EMAP-II specifically inhibited proliferation of HUVEC (a) but not fibroblasts (b).

2. Results

2.1. *EMAP-II specifically inhibits HUVEC cell proliferation*

HUVEC and human fibroblast cells were treated with EMAP-II for 2 h, 4 h, 1 day, 3 days and 5 days. The cells treated with EMAP-II elution buffer (as given in the Section 4), endostatin and 5-fluorouracil (5-FU) were used as controls. The results of the proliferation assay are shown in Fig. 1. Treatment with 10 μg of EMAP-II inhibited HUVEC proliferation in a time-dependent manner compared to non-treated controls (Fig. 1a). However, EMAP-II did not inhibit proliferation of human fibroblasts (Fig. 1b). Thus, the inhibitory activity of EMAP-II on cell proliferation appears to be specific for ECs when compared to fibroblasts.

2.2. *EMAP-II induces specific gene expression changes in HUVEC*

HUVEC cells treated with EMAP-II as described in the section 4 were studied using cDNA MA analysis. Of the 10,000 genes on the MA, 8382 genes were examined after correction for signal intensity and signal to background ratio at every spot. To examine overall changes in gene expression at different time points, scatter plots of basal expression (0 h) vs. expression at 0.5, 1, 2, 4 and 8 h were drawn on 8382 genes using BRB Array Tools (Fig. 2). The lines indicate 1:2 or 2:1 ratios between basal expression and expression at each time point. We observed the maximum number of genes changing their expression patterns between 0.5 and 2 h.

We observed changes in 69 genes using a twofold cutoff threshold (log ratio ≥ 2 or ≤ 0.5). The 69 genes, arranged in different categories according to their function, are shown in Table 1 with gene ID (used from Incyte Genomics, Inc.) and fold changes (linear scale) in gene expression at different time points compared to baseline.

Of the 69 genes, which demonstrated changes in response to EMAP-II treatment of HUVEC in our study, 18 genes have previously been reported to be modulated in EC including HUVEC when exposed to stimuli other than EMAP-II (Table 2). We observed

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