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Original article

Mechanism of matrix metalloproteinase axis-induced neointimal growth

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ABSTRACT

Tumor necrosis factor-α, platelet-derived growth factor, matrix metalloproteinases 9 and 2 have very important roles in neointimal hyperplasia, which develops after endovascular injury. However, the relationships among the four factors in inducing neointimal hyperplasia are unclear. Here, we used a mouse model of femoral arterial transluminal wire injury, and examined neointimal hyperplasia within the 28 days that followed the injury. We confirmed that the neointima kept growing during the 28 days, and found that expression of TNF-α and PDGF mRNAs in femoral arteries peaked within 24 h after injury. However, MMP9 mRNA expression peaked 7 days, and MMP2 mRNA expression peaked 28 days after injury. Then, we administered exogenous TNF-α or PDGF to the peri-femoral artery following an injury, and found that exogenous TNF-α led to significantly more neointimal hyperplasia during the first 2 weeks, and PDGF led to increased neointimal hyperplasia during the second 2 weeks after injury. We also used the model of femoral artery injury in MMP9- or MMP2-deficient (MMP9 −/− or MMP2 −/−) mice. We found that neointimal hyperplasia was reduced in MMP9 −/− mice during the first 2 weeks after injury, and neointimal hyperplasia was reduced in MMP2 −/− mice during the second 2 weeks after injury. When TNF-α or PDGF was administered to the peri-femoral artery immediately after injury, TNF-α did not promote neointimal hyperplasia in MMP9 −/− mice during the first 2 weeks after injury but did in MMP2 −/− mice, and PDGF did not promote neointimal hyperplasia in MMP2 −/− mice during the second 2 weeks after injury but did in MMP9 −/− mice. We used an in vitro system to treat vascular smooth muscle cells (VSMCs) with TNF-α or PDGF; TNF-α induced MMP9, but not MMP2, expression at a fast reaction speed, while PDGF induced MMP2, but not MMP9, expression at a slow reaction speed. Meanwhile, TNF-α induced VSMC migration in a MMP9-dependent manner, and PDGF induced VSMC proliferation in a MMP2-dependent manner. Taken together, our studies elucidated the axis of TNF-α-MMP9-VSMC migration and PDGF-MMP2-VSMC proliferation, both of which contributed to the mechanism of neointimal hyperplasia formation.

1. Introduction

Blood vessels are abundant in every vertebrate, and vascular disease can affect every organ and exacerbate other diseases [1]. Vascular neointimal hyperplasia is a key event in arteriosclerosis and several other vascular diseases [2,3]. Neointimal hyperplasia is an abnormal increase in the cell population within the innermost layer of an arterial wall; very similar proliferative growth occurs during normal physiological events, including closure of the arterial duct and involution of the uterus [4]. Underlying causes of neointimal hyperplasia include vascular smooth muscle cells (VSMCs) migration, proliferation, and apoptosis provoked by endovascular injury or perivascular injury [5,6]. According to these mechanisms above, the neointima consists mainly of VSMCs [7], although myofibroblasts and fibroblasts also reportedly contribute to the neointima [8].

VSMCs are critical to neointimal hyperplasia after injury; therefore, we have focused on those factors that can affect VSMCs. Once a blood vessel suffers an injury, large amounts of factors are provoked. Among them is TNF-α, a canonical inflammatory cytokine that accelerates neointimal hyperplasia by promoting VSMC migration and proliferation [9]. Additionally, PDGF [10,11], which can recruit VSMCs during embryonic blood vessel formation in mice, are important in neointimal hyperplasia [12]. PDGF induces vascular remodeling during development of pulmonary hypertension, and it plays a key role in a many blood vessel-related physiological or pathological changes [13]. However, apparently neither TNF-α nor PDGF promotes VSMCs growth directly.

In the process of VSMC migration and proliferation, the outer membranes of VSMCs need to be degraded by MMPs that are released from the extracellular matrix [4,14,15]. MMPs are primarily responsible for extracellular matrix remodeling, giving VSMCs an appropriate microenvironment for growth. Therefore, MMP-mediated degradation of the outer membrane of VSMCs may be the key inducer of VSMCs growth. However, a variety of research works revealed that proteolytic activity of MMPs controls availability of active molecules such as growth factors. In normal physiological conditions, MMPs activities are regulated at multiple levels, including gene transcription, activation of zymogens and interaction with specific inhibitors in order to limit MMPs activity [16]. So MMPs activity controls degradation of extracellular matrix and

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VSMCs outer membrane. Among the MMP protein family, two proteins-MMP9 and MMP2-are the most thoroughly studied. MMP9 stimulates VSMC migration and proliferation by organizing collagenous matrix and increase VSMCs attachment to gelatin [17], and MMP2 stimulates VSMC migration and proliferation by triggering ox-LDL induced activation of sphingomyelin/ceramide pathway and subsequent ERK1/2 activation and DNA synthesis [18], but apparently MMP9 and MMP2 are not simultaneously induced by any one stimulation [19]. TNF-α and PDGF can each independently induce either MMP9 or MMP2 expression [20,21], but it seems that TNF-α mainly induces MMP9, not MMP2 [22], expression and PDGF mainly induces MMP2 expression [23]. Above all, current evidence indicates that the activities of TNF-α, PDGF, MMP9, and MMP2 may be linked during induction of VSMC migration and proliferation. However, we do not know whether the putative links among TNF-α, PDGF, MMP9, and MMP2 also contribute to neointimal hyperplasia.

Recent reports indicate that chemokines and cytokines secreted from perivascular tissues play an important role in vascular diseases and neointimal hyperplasia [24–26], which indicated that factorsderived perivascular tissue influenced vascular remodeling. Also, perivascular administration of TNF-α enhanced neointimal hyperplasia formation [26], which implied a method of perivascular administration could be considered as a administration route and therapeutic strategy for study vascular diseases and treatment of neointimal hyperplasia. Although such perivascular administration has been used successfully for experimental applications after endovascular injury [27], further study is required.

Here, we used perivascular administration to examine the link between TNF-α and MMP9 and the link between PDGF and MMP2 in injury-induced neointimal hyperplasia. Our findings demonstrated that a time-dependent mechanism mediated vascular neointimal hyperplasia after endovascular injury.

2. Materials and methods

2.1. Animal model

In this experiment, the bilateral femoral arteries of 16-week-old C57BL/6N mice, MMP9 knockout mice (MMP9 −/−), MMP2 knockout mice (MMP2 −/−), MMP9 and MMP2 double knockout mice (MMP9/2 −/−) (Biocytogen Co., Ltd. Beijing, China) were subject to transluminal wire injury as described previously [7]. All mice were fed normal rodent chow and had ad libitum access to water.

2.2. Evaluation of neointimal hyperplasia

Femoral arterial tissues were excised 1, 3, 7, 14, or 28 days after endovascular injury. Each harvested tissue sample was fixed in formalin and embedded in paraffin. Each was then cut into section at 0.05-mm intervals to generate about 8–10 sections from each sample; each section was then stained with Elastica van Gieson, which result in a clear stain that facilitates visual determination of neointimal hyperplasia [28]. The area of neointima and media of the arteries were measured by SigmaPlot (Systat Sofeware Inc.), and the average of the neointima area/media area ratio was calculated as the neointimal hyperplasia index.

Other materials and methods are shown in Supplementary Materials and Methods.

3. Results

3.1. Neointima grows at several different growth rates after endovascular injury

In order to understand the growth speed of neointima during different periods of neointima hyperplasia, we first induced endovascular injury and examined neointimal hyperplasia at the 3rd, 7th, 14th, and 28th day after endovascular injury. We found that neointima underwent gradual growth until 28 days after injury (Figs. 1A and B). Next, we calculated the neointimal growth rate during four periods (0–3 days, 3–7 days, 7–14 days, and 14–28 days) following endovascular injury. We found that neointimal growth speed was fastest from 7 to 14 days after injury; during this period, the neointima grew by 42% of the total neointima. The next fast period was from 14 to 28 days after injury, the neointima grew by 34% of total neointima (Fig. 1C).

3.2. TNF-α, PDGF, MMP9, and MMP2 are induced at different periods after endovascular injury

Because the neointima apparently grew at different speeds during different periods after endovascular injury, we wondered whether TNF-α, PDGF, MMP9, and MMP2 have variable patterns of expression during different periods after endovascular injury. Expression of TNF-α and PDGF mRNA each peaked within 1 day after endovascular injury. Although expression of TNF-α and PDGF mRNA decreased after the first day, they also kept at a significant high level compared with 0d (Figs. 1D and E). At the same time, we also found that TNF-α mRNA expression was synchronized with the CD68 but not F4/80 mRNA expression, and may PDGF mRNA expression was synchronized with the CD54 but not CD31 mRNA expression. These results indicated TNF-α may come from the CD68 positive monocyte and PDGF may come from the CD54 positive leukocyte (Supplementary Fig. 1). These findings indicated the potential reigning role and sostenuto effect of TNF-α and PDGF in inducing neointimal hyperplasia. However, MMP9 and MMP2 mRNA expression peaked 7 and 28 days, respectively, after injury (Figs. 1F and G); as a matter of fact, MMP2 expression was decreasing after 28 days (data was not shown), and the mRNA peaks paralleled the peaks in protein levels (Fig. 1H). Therefore, MMP9 and MMP2 may played their respective role during different periods after endovascular injury.

3.3. TNF-α and PDGF play their respective roles in leading neointimal hyperplasia at different periods after endovascular injury

Both TNF-α and PDGF mRNA levels peaked within 1 day after endovascular injury; nevertheless, this finding did not address the mechanisms by which TNF-α and PDGF affected neointimal growth. To investigate these mechanisms, we administered TNF-α or PDGF-bb into the peri-femoral artery for 2 or 4 weeks immediately following endovascular injury. Administrated TNF-α promoted more neointimal growth during the first 2 weeks than the sham treatment (Figs. 2A a, b and B). The neointima was thicker in the TNF-α-treated group than in the sham-treated group 4 weeks after endovascular injury (Fig. 2C); nevertheless, when we calculated the neointimal growth speeds, we found that exogenous TNF-α induced 25% of the neointimal growth during the first 2-week period (0–2 W), and only 5% during the second 2-week period (2–4 W) (Fig. 2D). However, administrated PDGF-bb didn't promote neointimal growth in 2 weeks after injury (Fig. 2E), but promoted neointimal growth in the 4 weeks (Figs. 2F a, b and G). Ultimately, we found that the exogenous PDGF-bb induced 7% of the neointimal growth during the first 2-week period (0–2 W), and 19% during the second 2 weeks (2–4 W) (Fig. 2H).

Interestingly, the neointimal growth induced either by TNF-α or PDGF-bb could be inhibited by the MMP9/2 inhibitor (SB-3CT) (Figs. 2A c, B, C and E, F c, G). We found SB-3CT inhibited neointima/media ratio to 0.75 and 1.6 respectively in TNF-α administration group (Supplementary Fig. 2A), i.e.: SB-3CT inhibited 74% and 45% respectively in the first (0–2 W) and the second (2–4 W) two weeks after endovascular injury (Supplementary Fig. 2B). Similarly, SB-3CT inhibited neointima/media ratio to 0.6 and 1.7 respectively in PDGF-bb administration group (Supplementary Fig. 2C), i.e.: SB-3CT inhibited 77% and 35% respectively in the first (0–2 W) and the second (2–4 W) two weeks after endovascular injury (Supplementary Fig. 2D). This finding indicated that neointimal growth induced by TNF-α or by PDGF may depend upon MMP9/2.