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Full Length Article

A novel role of bone morphogenetic protein-7 in the regulation of adhesion and migration of human monocytic cells

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ABSTRACT

Background: Bone morphogenetic protein (BMP) 7 is abundant in atherosclerotic plaques and increases monocyte pro-coagulant activity by enhancing tissue factor (TF) expression. While several members of the BMP superfamily are able to serve as chemotactic agents for monocytes, the role of BMP-7 in regulation of monocyte motility is not known.
Aims: To assess the effect of BMP-7 on adhesive and migratory properties of human monocytes.
Methods: Chemokinesis, adhesion, and transendothelial migration of BMP-7-treated THP-1 cells and human monocytes were analysed using live-cell imaging, orbital shear, and Boyden chamber assays. Surface presentation of β2 integrins and phosphorylation status of Akt & focal adhesion kinase (FAK) were studied by flow cytometry and Western blot.
Results: High levels of BMP-7 protein were detectable in intimal regions of atherosclerotic plaques; BMP-7 significantly enhanced THP-1 and monocyte chemokinetic properties in vitro (1.21 + 0.01 and 1.76 + 0.21 fold increase in crawling distance, respectively). Under orbital shear, adhesion of monocytic cells to microvascular endothelial cell (MVEC) monolayers was also significantly increased by BMP-7 (3.89 + 1.56 and 2.57 + 0.97 fold over vehicle). Moreover, BMP-7 accelerated transendothelial migration of THP-1 cells and monocytes towards MCP-1 (5.91 + 0.88 and 2.96 ± 0.65 fold increase, respectively). BMP-7 enhanced cell surface presentation of β2 integrins in the active conformation. Observed effects were determined to be Akt and FAK dependent, as shown by pharmacological inhibition.
Conclusion: BMP-7 directly upregulates adhesion and migration of human monocytic cells via activation of β2 integrins, Akt, and FAK. Our findings suggest that BMP-7 may serve as a novel contributor to atherogenesis.

1. Background

The spectrum of monocyte function in health and disease is remarkably broad, ranging from innate immunity, antigen presentation [1], tumour angiogenesis [2] to wound healing [3] and thrombosis [4]. Immature monocytes as well as macrophages express non-specific scavenger receptor with high affinity to oxidized LDL (oxLDL) [5]. Internalisation of oxLDL by monocytes/macrophages in the plaque is widely accepted as a key process. During the early stages of atherogenesis monocyte extravasation, accumulation, and proliferation and engulfment of oxLDL in the lesions [6] lead to the formation of foam cells – lipid-laden macrophages that decrease plaque stability [7]. Adverse cardiovascular events continue to comprise the major cause of death worldwide [8]. Thus, it is critical to expand the existing knowledge of the processes that mechanistically contribute to atherogenesis.

Human monocytes express four β2 integrins, i.e. αM, αL, αX, and αD [9]: monocyte rolling on the extracellular matrix and endothelium was shown to be MAC-1 (αMβ2 integrin) dependent [10], whereas firm adhesion and extravasation require presentation of both MAC-1 and LFA-1 (αLβ2) on the surface of circulating monocytes in response to proinflammatory stimuli, e.g. cytokines, shear stress, and hypoxia [9,11,12]. Activation of Akt and FAK is pivotal in the upregulation of monocyte motility [13].

Bone morphogenetic proteins (BMPs) comprise a superfamily of proteins that were initially discovered as regulators of bone homeostasis. Recent data, however, suggest that BMPs are also involved in the pathogenesis of vascular disorders [14] and iron metabolism [15]. Moreover, BMP-7 has a potential protective role in renal diseases [16]. BMPs

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signal through a class of heterodimeric receptors sub-divided into type 1 and type 2 receptors [17]. Upon BMP binding to extracellular domain, phosphorylation of the cytoplasmic tail of type 1 receptor occurs and triggers activation of the downstream signalling proteins SMAD1/5/8 and SMAD4 [18]. SMAD4 binds to *cis*-acting elements in promoter regions of several genes and, in concert with other DNA binding proteins, upregulates gene transcription [18]. Apart from the canonical SMAD pathways, BMPs convey their effects via a variety of signalling kinases, including Akt and mitogen-activated protein kinases (MAPKs), e.g. extracellular signal-regulated kinase (ERK) 1/2, p38, and Janus N-protein kinase [19,20].

We and others have previously shown that BMP-2, -4, and -7 are present in atherosclerotic lesions [21,22]. BMPs are known to serve as potent chemoattractants for leukocytes [22–24]. Plasma levels of BMP-2 positively correlate with the extent of atherosclerotic burden in type 2 diabetes [25]. In a murine model, BMP-2 increases arterial calcification [26]. In contrast to BMP-2, BMP-7 is abundant in lipid-rich plaques [27] and impedes vascular calcification in a murine model [28]. Monocytes express functional BMP receptors (BMPRs) and, following exposure to BMPs, upregulate the expression of pro-coagulant tissue factor (TF) and inflammatory cytokines [21,27], thus contributing to destabilisation of lipid-rich plaques.

In the present study, we sought to investigate whether direct exposure of monocytes to BMP-7 affects their adhesion and migration, key events in early atherosclerosis.

2. Materials and Methods

2.1. Reagents

Human monocytic cell line THP-1 (ATCC, Manassas, VA, USA) was grown according to the supplied protocol. Primary human microvascular endothelial cells (MVEC; Cell Systems, Kirkland, WA, USA) were grown according to the manufacturer's instructions. Human recombinant BMP-7 and monocyte chemoattractant protein (MCP-1) and Noggin purified from eukaryotic cells, were purchased from R&D Systems (Minneapolis, MN, USA). Dorsomorphin was from Stemcell (Cambridge, UK). β2 integrin high affinity confirmation antibody (clone mAb24) and rabbit polyclonal antibody to human BMPR2 were from Abcam (Cambridge, UK). Antibodies to pAkt (Thr308), pFAK (Tyr397), total Akt, and total FAK were from Cell Signalling (Danvers, MA, USA). Fluorochrome- and HRP-conjugated secondary antibodies, DAPI and DRAQ5 were from Life Technologies (Carlsbad, CA, USA). Calcein-AM and transwell inserts (3.0 μm pore size, for 24-well plates) were from BD

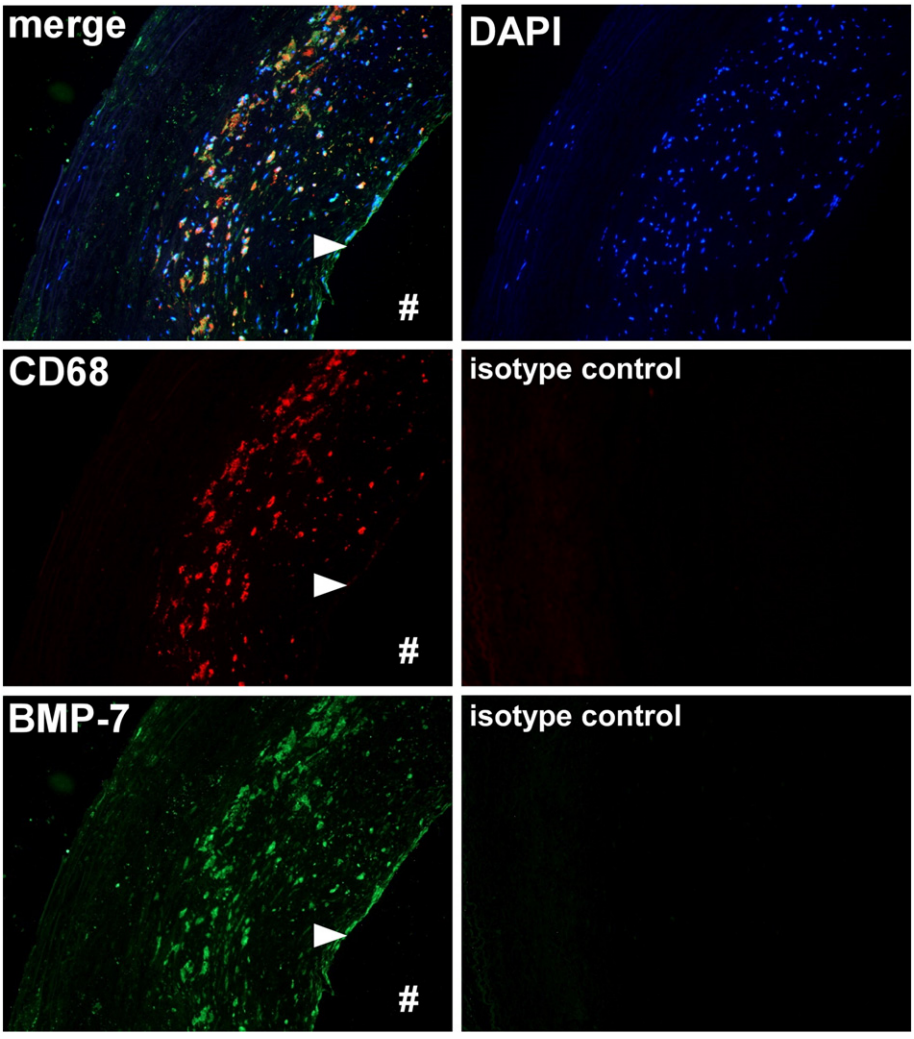

Fig. 1. Areas of atherosclerotic plaque positive for BMP-7 (green) are enriched with infiltrating macrophages (visualized with α-CD68 immunostaining, red). Note the abundance of BMP-7 staining along the intimal lining of the lesion (arrow), lumen marked by #. Blue staining in the merged panel: DAPI-visualized nuclei.