Breast cancer-derived extracellular vesicles stimulate myofibroblast differentiation and pro-angiogenic behavior of adipose stem cells

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Abstract

Adipose-derived stem cells (ASCs) are abundantly present in the mammary microenvironment and can promote breast cancer malignancy by differentiating into myofibroblasts. However, it remains largely unclear which role tumor-derived extracellular vesicles (TEVs) play in this process. Here, we used microfabricated, type I collagen-based 3-D tissue culture platforms to investigate the effect of breast cancer cell-derived TEVs on ASCs myofibroblast differentiation and consequential changes in extracellular matrix remodeling and vascular sprouting. TEVs collected from MDA MB-231 human metastatic breast cancer cells (MDAs) promoted ASC myofibroblast differentiation in both 2-D and 3-D cultures as indicated by increased alpha smooth muscle actin (α-SMA) and fibronectin (Fn) levels. Correspondingly, TEV-treated ASCs were more contractile, secreted more vascular endothelial growth factor (VEGF), and promoted angiogenic sprouting of human umbilical vein endothelial cells (HUVECs). These changes were dependent on transforming growth factor beta (TGF-β)-related signaling and tumor cell glutaminase activity as their inhibition decreased TEV-related myofibroblastic differentiation of ASCs and related functional consequences. In summary, our data suggest that TEVs are important signaling factors that contribute to ASC desmoplastic reprogramming in the tumor microenvironment, and suggest that tumor cell glutamine metabolism may be used as a therapeutic target to interfere with this process.

Introduction

Excessive fibrotic remodeling of the stroma, termed desmoplasia, is a hallmark of breast cancer that is mediated by myofibroblasts and correlates with an advanced, invasive phenotype and worse clinical prognosis [1–3]. Myofibroblasts are highly contractile cells that contain alpha smooth muscle actin (α-SMA)-positive stress fibers [4] and are able to deposit and remodel key fibrillar components of the extracellular matrix (ECM) including type I collagen and fibronectin [5,6]. The resulting compositional, structural, and mechanical changes of the ECM directly impact tumor cell aggressiveness [7–9]. Increasing evidence also suggests that myofibroblasts play a critical role in regulating tumor angiogenesis, *i.e.*, the formation of new blood vessels from a pre-existing vasculature necessary for tumor growth and metastasis [10–12]. More specifically, myofibroblasts can promote vascular sprouting directly and indirectly through their secretion of key pro-angiogenic factors including vascular endothelial growth factor (VEGF) and their ECM remodeling capability, respectively [13].

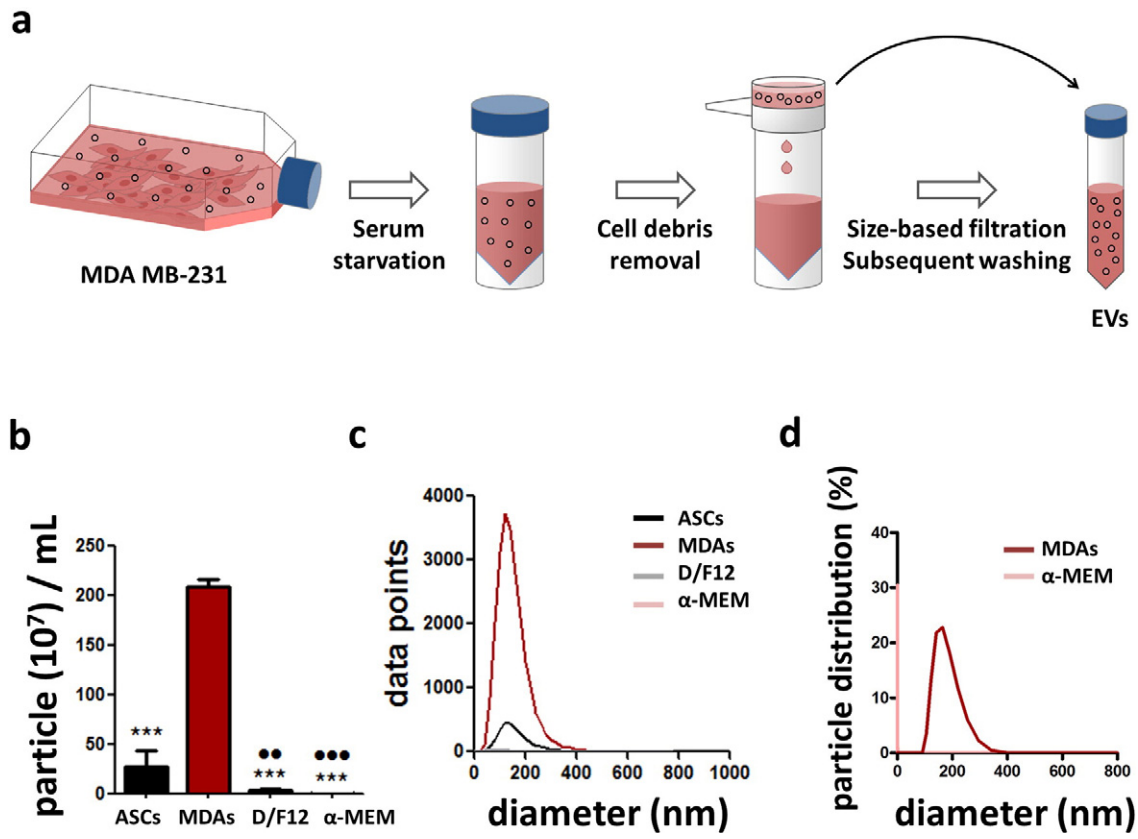

Fig. 1. Collection and characterization of extracellular vesicles (EVs). a) Subconfluent MDA MB-231 (MDAs) were subjected to serum starvation for 7–12 h. Media were harvested, centrifuged to remove cell debris, and then filtered to enrich for EVs. This EV fraction was then used for subsequent experiments and analyses. Control media were prepared by placing serum-free media in the incubator and processing it similarly. b) Concentration of particles in the blank control media *vs.* ASC- and MDA-derived EVs as measured by NanoSight. ***$p < 0.001$ *vs.* MDAs, ●● $p < 0.01$ *vs.* ASCs, and ●●● $p < 0.001$ *vs.* ASCs c) Size distribution of particles in the blank control media *vs.* ASC- and MDA-derived EVs as measured by NanoSight. d) Particle size distribution in blank control media *vs.* MDA-conditioned media as measured by Zetasizer.

While fibroblasts and bone marrow-derived mesenchymal stem cells are typically discussed as the main cellular source of myofibroblasts [14,15], adipose-derived stem cells (ASCs) may be similarly important [16]. ASCs are abundantly contained in mammary fat, but can also circulate in the blood stream and thus be recruited to mammary tumors from distant sites [17]. In fact, breast cancer-associated ASCs can differentiate into myofibroblasts [18] as detected by increased expression of α-SMA [19], greater contractility [20], and elevated deposition of fibrillar ECM proteins [21,22]. Additionally, ASCs have also been shown to modulate angiogenesis through various mechanisms. For example, ASCs secrete pro-angiogenic factors such as VEGF, associate perivascularly with blood vessels, and provide physical ECM guidance cues that promote endothelial sprouting [13,23,24]. Collectively, these changes render the tumor microenvironment more permissive for further progression towards malignancy [22]. However, the signaling mechanisms by which tumors transform ASCs into myofibroblasts and the resulting consequences on endothelial cell invasion and subsequent tumor angiogenesis remain poorly understood.

Tumor cell-derived extracellular vesicles (TEVs) including exosomes and microvesicles (MVs) are increasingly recognized for their role in tumorigenesis and appear to also play a role in myofibroblast differentiation, but their effects on ASCs remain unclear. Exosomes are released from late endosomal multivesicular bodies [25], whereas MVs are generated by outward budding and pinching off of the plasma membrane and at greater levels by more malignant cancer cells [26]. Once thought to be cellular debris, these TEVs contain a variety of signaling molecules that prime the host microenvironment for tumor progression [27–30]. More specifically, TEVs not only facilitate the evasion of immune responses and drug therapy, but also support the establishment of pre-metastatic niches [31–33]. TEVs have also been shown to promote