Mast Cells Are Directly Activated by Cancer Cell–Derived Extracellular Vesicles by a CD73- and Adenosine-Dependent Mechanism☆

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Abstract

We have recently shown that mast cells (MCs), which constitute an important part of the tumor microenvironment (TME), can be directly activated by cancer cells under conditions that recapitulate cell to cell contact. However, MCs are often detected in the tumor periphery rather than intratumorally. Therefore, we investigated the possibility of MC activation by cancer cell–derived extracellular vesicles (EVs). Here we show that exposure of MCs to EVs derived from pancreatic cancer cells or non–small cell lung carcinoma results in MC activation, evident by the increased phosphorylation of the ERK1/2 MAP kinases. Further, we show that, similarly to activation by cancer cell contact, activation by EVs is dependent on the ecto enzyme CD73 that mediates extracellular formation of adenosine and on signaling by the A3 adenosine receptor. Finally, we show that activation by either cell contact or EVs upregulates expression of angiogenic and tissue remodeling genes, including IL8, IL6, VEGF, and amphiregulin. Collectively, our findings indicate that both intratumorally localized MCs and peripheral MCs are activated and reprogrammed in the TME either by contact with the cancer cells or by their released EVs.

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Introduction

Cells of the immune system infiltrate tumors and comprise an important constituent of the tumor microenvironment (TME). Moreover, immune cells exert both anti- and protumorigenic effects, thus acting as a double-edged sword [1–3]. Such is the case of the mast cells (MCs), immune cells that are best known for their involvement in allergic reactions; however, cumulative data indicate their likewise important role in tumorigenesis [4,5]. Indeed, MCs infiltrate a large number of tumors, and depending on the circumstances, which still need to be resolved, MCs may function to promote or restrict tumor growth and invasiveness [6–9]. MCs perform their function by releasing multiple inflammatory mediators [10,11]. The latter, including vasoactive amines, such as histamine, proteases, chemokines and cytokines [11–13], have the potency to promote or inhibit malignancy [14]. Hence, by releasing chemokines and initiating an immune response, MCs may lead to tumor eradication. However, by the release of angiogenic factors, matrix metalloproteinases (MMPs), and immunosuppressive cytokines, MCs may provide the tumor with a supportive environment [15–19]. Therefore, MCs can orchestrate tumor growth and define its projections [14,20].

We have recently demonstrated that MCs can be directly activated by cancer cells [21]. Exposure of model human mast cell lines (i.e., HMC-1 and LAD-2 cells), as well as primary mouse bone marrow–derived MCs (BMMCs), to a number of cancer cell line–derived membranes, conditions that recapitulate cell contact–mediated activation [22,23], resulted in MC activation, thus demonstrating direct activation of MCs by cell contact with cancer cells. Consistent with this notion, MCs formed synapses with the cancer cells in co-culture [21]. However, in many occasions, MCs are detected only in the tumor periphery,

eliminating cell-to-cell contact as a mechanism of MC manipulation by cancer cells [24,25]. This clinical observation prompted us to explore the possibility that MCs could also be activated by extracellular vesicles (EVs) that are released by the cancer cells. We based this assumption on clinical observations that have clearly demonstrated release of EVs by tumor cells [26]. Furthermore, proteomics profiling of tumor derived EVs content has indicated significant differences in comparison to the content of EVs released by normal cells [27]. Hence, cancer cells actively release EVs into their microenvironment, and by influencing their neighboring cells, they contribute to cancer progression and immune modulation [27]. Consistent with this notion, here we demonstrate that EVs derived from pancreatic and lung cancer cells stimulate ERK1/2 MAP kinase signaling in MCs. Moreover, in a similar manner to activation by cell contact [21], activation by cancer cell–derived EVs also involves autocrine formation of adenosine and activation of the adenosine A3 receptor (A3R), leading to the upregulation of tissue remodeling genes.

Materials and Methods

Materials and Cell Culture

Antibodies used included anti–phospho-ERK1/2 (Sigma-Aldrich, St. Louis, MO), anti-ERK2 (Santa-Cruz Biotechnology, Santa Cruz, CA), and HRP-conjugated goat anti-mouse and anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). The A3R antagonist 9-chloro-2-(2-furanyl)-5-((phenylacetyl)amino)-[1,2,4]triazolo[1,5-c]quinazoline (MRS1220) and adenosine 5′-(α,β-methylene) diphosphate (APCP) were purchased from Sigma-Aldrich (St. Louis, MO). Go6976, GF109203X, LY294002, SB 203580, and U0126 were purchased from A.G. Scientific Inc.

LAD-2 cells (a kind gift of Dr. D. Metcalfe, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) were cultured in StemPro (GIBCO, Carlsbad, CA), supplemented with 100 ng/ml hrSCF (Pepro-tech, Rocky Hill, NG), 2 mM glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin
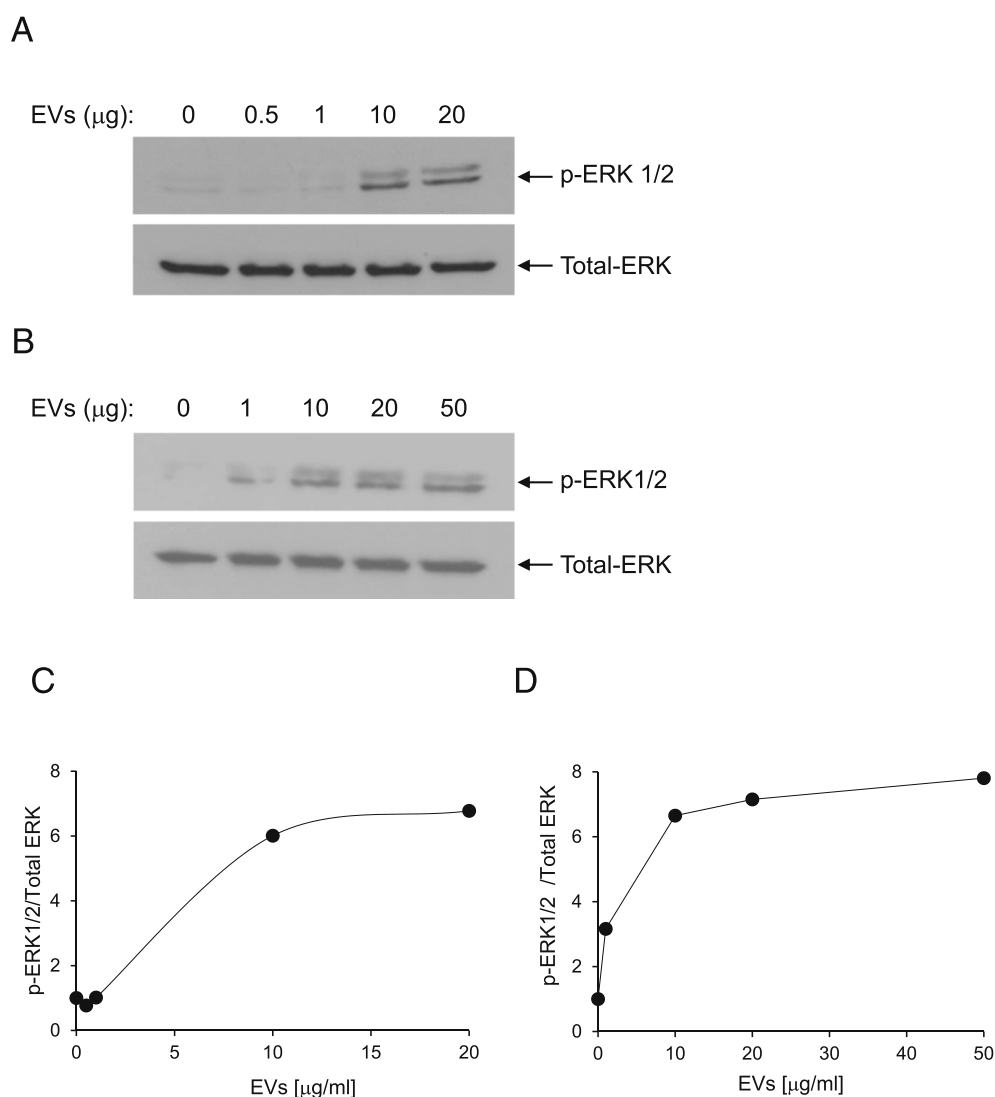

Figure 1. Stimulation of ERK 1/2 phosphorylation by cancer cell derived EVs. LAD-2 cells (1×10^6 cells/ml) were incubated for 1 minute with the indicated concentrations of EVs derived from either H1299 cells (A and C) or MIA PaCa-2 cells (B and D). Cell lysates were resolved by SDS-PAGE and immunoblotted with anti—p-ERK1/2 antibodies, followed by reprobing with anti—total ERK2 as indicated. The intensities of the bands corresponding to phospho-ERK1/2 and total ERK2 were quantified by densitometry using Image-J software, and the relative pixel densities (phosphorylated/total) were calculated. Representative blots are shown.

(Biological Industries, Bet-Haemek, Israel) at 37°C in a humidified incubator with 5% CO_2. HMC-1 cells (a kind gift from Dr. J. Butterfield, Mayo Clinic, Rochester, MN) were cultured in RPMI supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin and 12.5 U/ml nystatin (Biological Industries, Bet-Haemek, Israel). The human pancreatic cancer cell line MIA PaCa-2 and the non–small cell lung carcinoma (NSCLC) cell line H1299 were grown in RPMI supplemented with 10% FCS, 2 mM glutamine, 100 μg/ml streptomycin, 100 U/mL penicillin, and 12.5 U/ml nystatin (Biological Industries, Bet-Haemek, Israel).

Preparation of EVs

EVs were isolated from conditioned media from H1299 or MIA PaCa-2 cells by differential centrifugation. In brief, cell supernatants were harvested and centrifuged at $300 \times g$ for 10 minutes to eliminate cells and at $4500 \times g$ for 5 minutes to remove large debris. EVs were pelleted by ultracentrifugation at $100,000 \times g$ for 70 minutes at 4°C (Beckman Ti70 rotor; Beckman Centrifuge); washed with PBS; and, after additional centrifugation at $100,000 \times g$ for 70 minutes at 4°C, resuspended in PBS. Total protein concentration was measured at 280 nm using a Nanodrop spectrophotometer (Thermo Scientific Nano-Drop 1000; Thermo Fisher Scientific).

Activation of MCs

LAD-2 cells were washed twice with Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5.6 mM glucose, 1 mg/ml BSA, 20 mM Hepes pH 7.4) and resuspended to 1×10^6 cells/ml in the same buffer. Cells were then incubated at 37°C for the desired time periods with EVs isolated from the conditioned media of the human pancreatic cancer MIA PaCa-2 or the NSCLC H1299 cell lines. Reactions were terminated by placing the tubes on ice followed by a brief spin ($14,000 \times g$, 20 seconds) at 4°C. In experiments that included inhibitors, the cells were preincubated with the desired inhibitor or corresponding vehicle for 30 minutes at 37°C prior to stimulation with EVs.

EV Uptake

EVs derived from H1299 cells were labeled with PKH67 Green Fluorescent Cell Linker Kit for General Cell Membrane Labelling (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's protocol.

Quantitative Real-Time PCR

Total cellular RNA was extracted using the PerfectPure RNA Purification System (5′ prime) according to the manufacturer's protocol. cDNA was generated using 1 μg of total RNA and the high-capacity reverse transcriptase (Applied Biosystems) in a total volume of 20 μl. Quantitative real-time PCR was performed in an ABI Prism 7900 Sequence Detection System (Applied Biosystems) using the SYBR Green Real-Time PCR Kit (Applied Biosystems) and 20-60 ng of DNA in a total volume of 10 μl according to the manufacturer's instructions. Relative mRNA levels of the tested genes were calculated by the two standard curves method using HPRT RNA as a reference. The following primers were used:

IL8:
Forward: 5′-AGCTGGCCGTGGCTCTCT-3′.
Reverse: 5′-CCTTGGCAAAACTGCACCTT-3′;
IL6:

Forward: 5′-CAGAAAACAACCTGAACCTTCCA-3′;
Reverse: 5′-AAGTCTCCTCATTGAATCCAGATTG-3′;
AREG:
Forward: 5′-GATACTCGGCTCAGGCCATTAT-3′;
Reverse: 5′-CAAATCCATCAGCACTGTGGTC-3′;
VEGF:
Forward: 5′-TGCCCGCTGCTGTCTAAT-3′;
Reverse: 5′-TCTCCGCTCTGAGCAAGG-3′,
HPRT:
Forward: 5′-GACTTTGCTTTCCTTGGTCAGG-3′;
Reverse: 5′-TCCTTTTTCACCAGCAAGCTTG-3′ .

The data are presented as fold change in mRNA levels in treated vs. control cells.

Protein Extraction and Western Blot Analysis

Cell pellets were lysed by the addition of a lysis buffer [Buffer A comprising: 150 mM sucrose, 80 mM β-glycerophosphate, 2 mM EDTA, 2 mM EGTA, 2 mM $NaVO_3$, 10 mM sodium pyrophosphate (NaPPi), 1% Triton X-100, 1 mM PMSF and protease inhibitor cocktail (Roche)] and incubation for 20 minutes on ice. The lysates were then centrifuged for 15 minutes at $14,000 \times g$. Whole cell lysates, normalized according to protein content, were resolved by SDS-PAGE and immunoblotted with anti-phosphoERK1/2 antibodies followed by reprobing with anti–total ERK2 antibodies. Immunoreactive bands were visualized by enhanced chemiluminescence method (ECL) according to standard
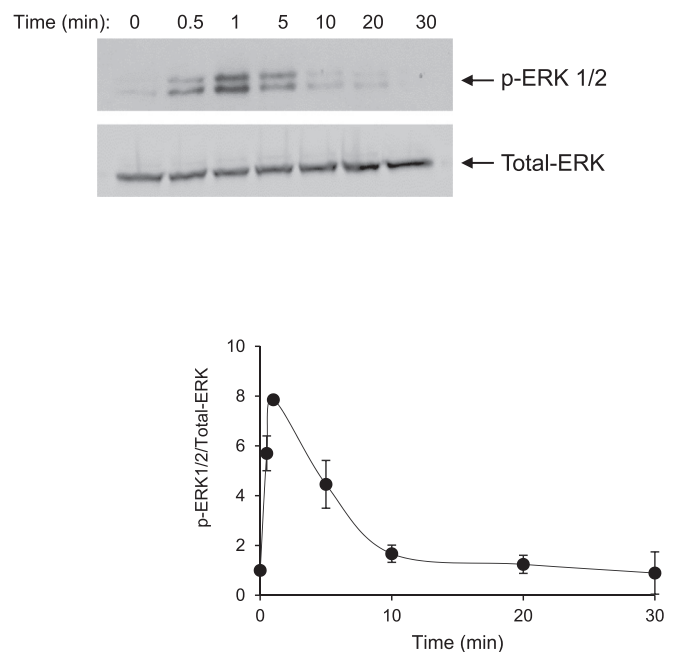

Figure 2. Kinetics of ERK 1/2 phosphorylation stimulated by H1299 cell–derived EVs. LAD-2 cells (1×10^6 cells/ml) were incubated for the indicated time periods with 50 μg/ml of EVs derived from H1299 cells. Cell lysates were resolved by SDS-PAGE and immunoblotted with anti–p-ERK1/2 antibodies followed by reprobing with anti–total ERK2 as indicated. Representative blots are shown. The intensities of the bands corresponding to phospho-ERK1/2 and total ERK2 were quantified by densitometry using Image-J software, and the relative pixel densities (phosphorylated/total) were calculated. Data presented are mean ± SEM of two independent experiments.

procedures. The intensities of the bands were quantified by Image-J software, and the relative pixel densities (phosphorylated/total) were calculated.

Data Presentation

Statistical analysis was performed using the two-tailed Student *t* test for unpaired data. Data are presented as mean ± SEM of the listed independent experiments.

Results

Effect Of Cancer Cell-Derived EVs On MC Activation Status

To determine if cancer cell–derived EVs can directly activate MCs, we isolated EVs from the conditioned media of the human pancreatic cancer cell line MIA PaCa-2 and from the NSCLC H1299 cells, which we have previously shown to activate directly human MCs by cell-to-cell contact [21]. We then examined if EVs derived from these cells can stimulate ERK1/2 phosphorylation in LAD-2 human MCs. We have chosen ERK1/2 phosphorylation as a reporter for MC activation because this process occurs in response to MC triggering by a variety of stimuli, including activation via the FcεRI, the high-affinity receptor for immunoglobulin E (IgE) [28], activation by multiple ligands that bind to G protein−coupled receptors (GPCRs) [22], activation via Toll-like receptors [29], and activation by cell contact with cancer cells including the MIA PaCa-2 and H1299 cancer cells [21]. Indeed, results showed that exposure to either MIA PaCa-2− or H1299-derived EVs stimulates phosphorylation of ERK1/2 in a dose-dependent manner (Figure 1).

Focusing on MC activation by H1299 NSCLC cells, we next examined the kinetics of ERK1/2 phosphorylation and found that phosphorylation was rapid and transient, reaching a maximal response by 1 minute and decaying thereafter (Figure 2). Hence, ERK1/2 phosphorylation by cancer cell−derived EVs displayed a similar kinetics to activation by cancer cell−derived membranes, which we have previously shown to be rapid and transient [21]. Notably, similar results were obtained using HMC-1 cells, another human mast cell line, indicating that activation by EVs was independent of the MC type (not shown).

MCs also took up the EVs. However, the kinetics of EV uptake was significantly slower than the kinetics of EV-induced signaling,
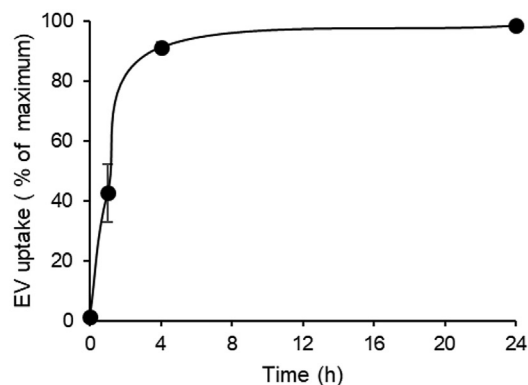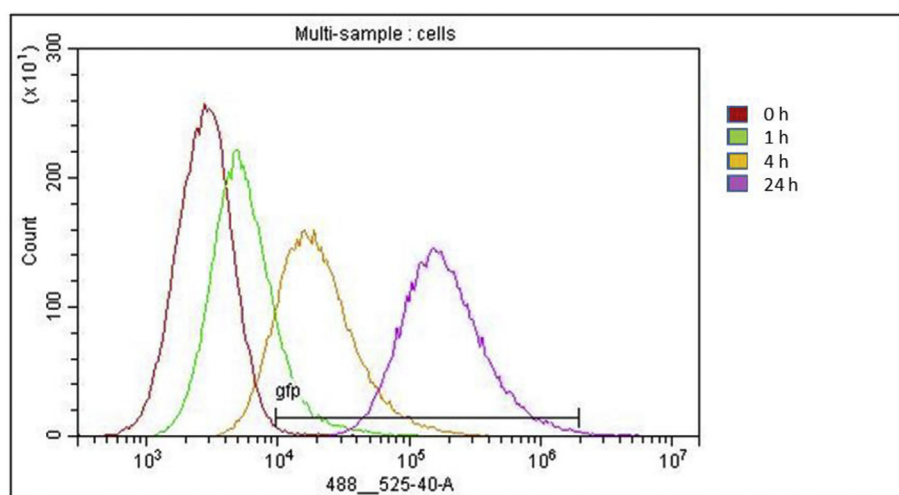

Figure 3. Kinetics of EV uptake. HMC-1 cells (2 × 10⁵ cells/ml) were incubated at 37°C for the indicated time periods with 10 μg of PKH67−labeled EVs derived from H1299 cells. Cells were then washed with phosphate-buffered saline and analyzed by flow cytometry using a CYTOFLEX flow cytometer (Beckman Coulter).
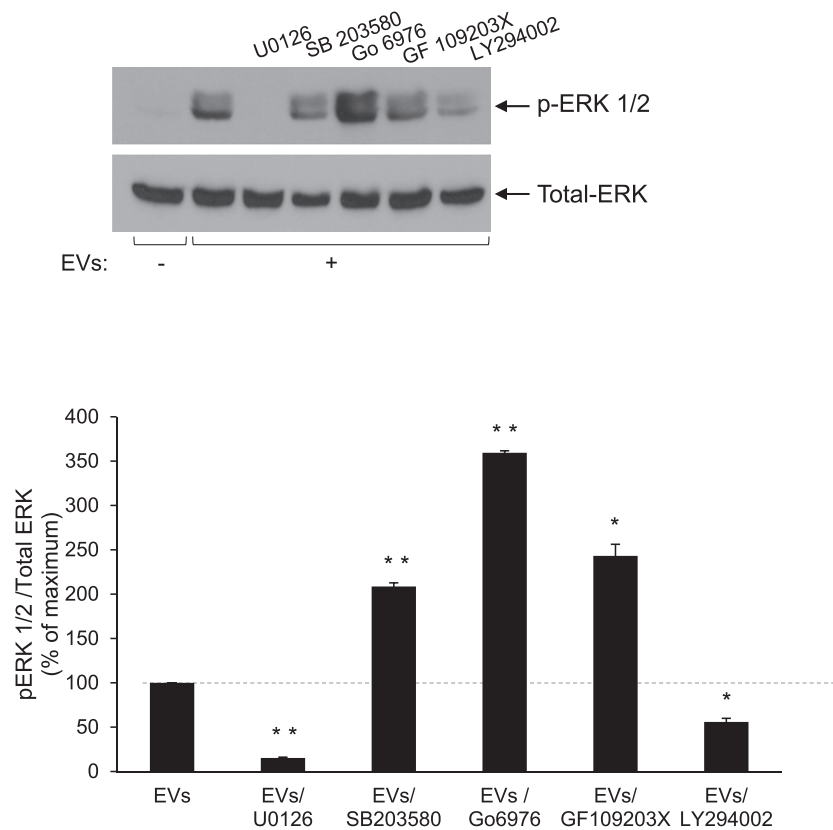
Figure 4. Kinase inhibitor profiling of EV-mediated activation of ERK 1/2 phosphorylation. LAD-2 cells (1 × 10^6 cells/ml) were either left untreated or incubated for 30 minutes with either vehicle or 3 μM of U0126, 10 μM of SB203580, 1 μM of Go6976, 1 μM of GF109203X, or 10 μM of LY294002, as indicated, followed by 1-minute incubation with 50 μg/ml of EVs derived from H1299 cells. Cell lysates were resolved by SDS-PAGE and immunoblotted with anti—p-ERK1/2 antibodies followed by reprobing with anti—total ERK2 as indicated. The intensities of the bands corresponding to phospho-ERK1/2 and total ERK2 were quantified by densitometry using Image-J software, and the relative pixel densities (phosphorylated/total) were calculated. Representative blots are shown. Data presented as percentage of maximal response are mean ± SEM of three independent experiments. Maximal response (EVs vs. untreated) was 8.6-fold increase, P = 5.8E-3. **P (EVs/U0126 vs. EVs) = 8.33E-6; **P (EVs/SB203580 vs. EVs) = .0016; **P (EVs/Go6976 vs. EVs) = 5.9E-4; *P(EVs/GF109203X vs. EVs) = 0.03; *P (EVs/LY294002 vs. EVs) = .03.

reaching maximum uptake following 4 hours of incubation (Figure 3). Therefore, these results strongly suggest that EV uptake and ERK1/2 phosphorylation are distinct processes that may elicit different responses. We envision that ERK1/2 phosphorylation is likely to involve contact between the MC and the EV membrane, recapitulating cell contact—mediated activation, while EV uptake may lead to other cellular responses.

Effect Of Inhibition of Phosphatidylinositol 3 kinase (PI3K) Activity On EV-Stimulated ERK1/2 Phosphorylation. We also analyzed the drug sensitivity of ERK1/2 phosphorylation, which we have previously shown to partially depend on the activity of PI3K(s), when activated by cancer cell derived membranes [21]. Indeed, in a similar manner, ERK1/2 phosphorylation stimulated by H1299 cell—derived EVs was partially (by 30%) inhibited by LY294002, a pan inhibitor of PI3Ks, while it was completely abrogated by U0126, an inhibitor of the MEK kinase that resides upstream of ERK1/2 (Figure 4). In contrast, neither Go6976 or GF109203X, two potent inhibitors of protein kinase C, nor SB203580, an inhibitor of the p38 MAP kinase, inhibited ERK1/2 phosphorylation (Figure 4). In fact, inhibition of either protein kinase C or p38 MAP kinase led to a significant increase in phosphorylation of ERK1/2, implying their involvement in negative regulation of EV-stimulated ERK1/2 signaling (Figure 4).

Effect Of Inhibition of Extracellular Adenosine And A3 Adenosine Receptor-Mediated Signaling On EV-Stimulated ERK1/2 Phosphorylation. Because cell contact—mediated activation of ERK1/2 was associated with autocrine signaling of adenosine [21], we next asked if EV-stimulated phosphorylation of ERK1/2 was sensitive to inhibition by APCP, a selective inhibitor of CD73, the ecto-5′-nucleotidase that mediates autocrine formation of adenosine [30]. Indeed, APCP significantly inhibited EV-induced ERK1/2 phosphorylation (Figure 5). Further, because cell contact—mediated activation of ERK1/2 was found to be mediated by the A3R [21], we also analyzed the impact of MRS1220, a specific antagonist of the human A3R, on this process. The results of these experiments clearly demonstrated that, similarly to cell contact—mediated activation of the MCs, ERK1/2 phosphorylation stimulated by EVs was significantly (by 65%) inhibited by MRS1220 (Figure 5). Therefore, collectively, these results implicated autocrine adenosine signaling in contributing to phosphorylation of the ERK1/2 MAP kinases and suggested a common underlying mechanism for MC activation by contact with cancer cells or with their derived EVs.

Effect Of EVs-Mediated Activation Of MCs On The Expression Of Tissue Remodeling Genes. Previously, we have shown that MC activation by Cl-IBMECA, a specific agonist of the A3R, leads to
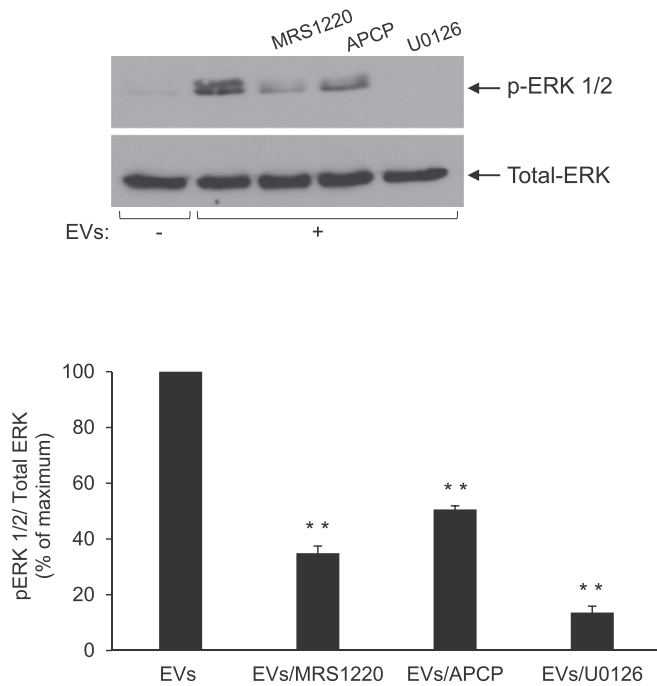
Figure 5. EVs stimulate ERK1/2 phosphorylation in MCs by a CD73- and A3R-dependent mechanism. LAD-2 cells (1×10^6 cells/ml) were incubated for 30 minutes in the absence or presence of 5 μM APCP, 100 nM of MRS1220, or 3 μM of U0126, as indicated. Cells were then left untreated or treated for 1 minute with 50 μg/ml of EVs derived from H1299 cells. Cell lysates were resolved by SDS-PAGE and immunoblotted with anti—p-ERK1/2 antibodies followed by reprobing with anti—total ERK2 as indicated. The intensities of the bands corresponding to phospho-ERK1/2 and total ERK2 were quantified by densitometry using Image-J software, and the relative pixel densities (phosphorylated/total) were calculated. Representative blots are shown. Data presented as percentage of maximal response are mean ± SEM of three independent experiments. **P(EVs/MRS1220 vs. EVs) = 1.8E-3; **P(EVs/APCP vs. EVs) = 5.4E-3; **P(EVs/U0126 vs. EVs) = 5.5E-3.

Figure 6. NSCLC-derived EVs or membranes upregulate tissue remodeling genes in MCs. LAD-2 cells (1×10^6 cells/ml) were left untreated (control, CNTR) or incubated for 3 hours with 50 μg/ml of either H1299-derived EVs (A) or membranes derived from H1299 cells (B). The relative expression levels of IL8, AREG, IL6, and VEGF were determined by quantitative real-time PCR as described under "Materials and Methods." Results are presented as fold increase relative to untreated cells. Data are means ± SEM derived from three independent experiments. (A) **P(IL8 vs. CNTR) = 1E-3; **P(AREG vs. CNTR) = 1.3E-3; *P(VEGF vs. CNTR) = 0.047; (B) **P(IL8 vs. CNTR) = 2.7E-3; **P(AREG vs. CNTR) = 3.2E-3; **P(VEGF vs. CNTR) = 1.5E-3.

upregulation of tissue remodeling genes [31]. Specifically, we showed that such treatment results in the upregulation of angiogenic genes and upregulation of amphiregulin (AREG), a ligand of the EGF receptor that serves as a potent growth factor of lung cells [31,32]. Because EV-stimulated activation of MCs was associated with adenosinergic signaling by the A3R, we next investigated whether activation by the cancer cell—derived EVs was linked with changes in gene expression. To this end, we quantified the amount of mRNA encoding VEGF, IL8, IL6, and AREG, which we have previously shown to be upregulated by Cl-IBMECA [31], in resting as compared with EV-activated cells. Consistent with our expectation, a clear increase in the expression of these tissue remodeling genes was noted (Figure 6A). Furthermore, a similar increase was detected in cells that were activated by membranes derived from the H1299 cells (Figure 6B), supporting further the notion that autocrine adenosine signaling contributes to MC activation by contact with cancer cells or by their derived EVs. In sharp contrast, neither exposure to H1299-derived membranes nor to their released EVs has stimulated MC degranulation, as evidenced by the failure of the activated MCs to release histamine (not shown).
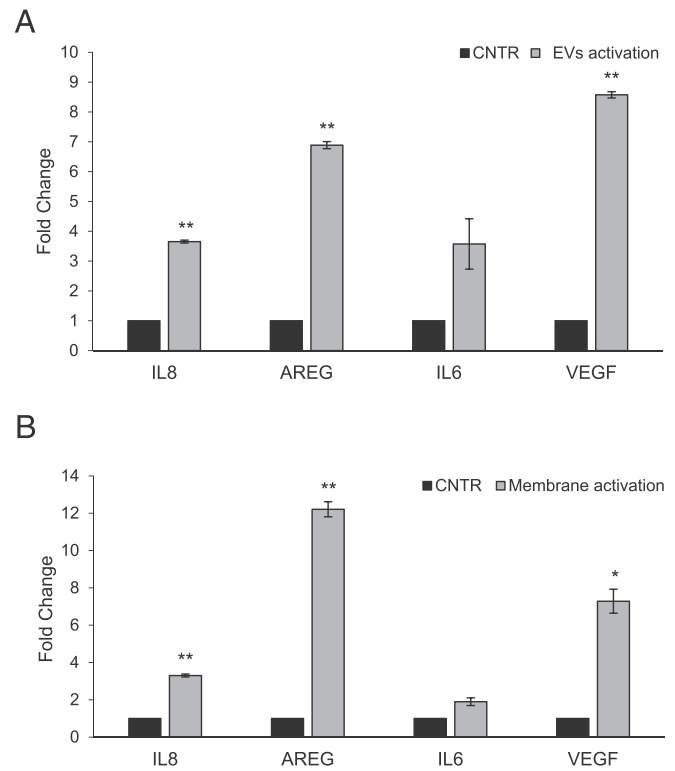
Effect Of Inhibition of A3 Adenosine Receptor-Mediated Signaling On The Upregulation Of Tissue Remodeling Genes.   To further assess the contribution of A3R signaling to EV-induced upregulation of tissue remodeling genes, we also investigated the impact of A3R inhibition on this process. Results of these experiments demonstrated significant inhibition substantiating the involvement of A3R signaling in remodeling gene upregulation (Figure 7). Interestingly, while upregulation of IL8, IL6, and VEGF was inhibited by 40% by the presence of the A3R antagonist MRS1220, upregulation of AREG was inhibited by 80% (Figure 7). These results implicate additional signaling pathways that are concomitantly activated in the EV-triggered cells in the upregulation of the angiogenic genes, while upregulation of AREG is primarily mediated by the autocrine signaling of the A3R.

We also assessed the contribution of ERK1/2 signaling, which resides downstream of the A3R, by analyzing the impact of the MEK inhibitor U0126 on the upregulation of the tissue remodeling genes. Again, while all genes were significantly inhibited by the MEK inhibitor, we noted differences in their drug sensitivity, whereby both VEGF ad IL6 displayed increased sensitivity (60% to 75% inhibition), while IL8 and AREG were more resistant (20% to
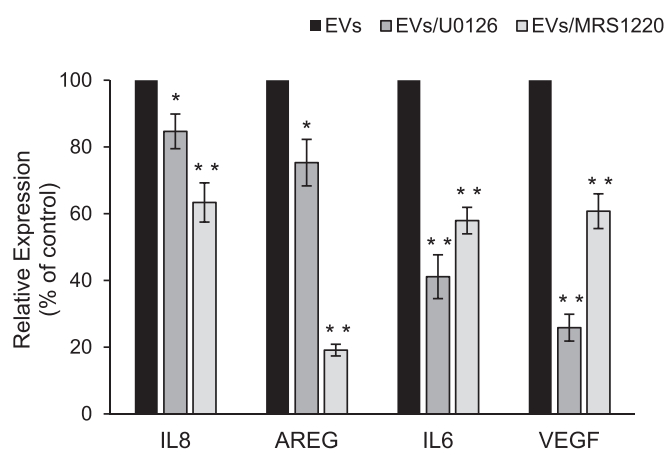
Figure 7. NSCLC-derived EVs' upregulation of gene expression is partially dependent on A3R signaling and the ERK1/2 pathway. LAD-2 cells (1×10^6 cells/ml) were incubated for 30 minutes either with vehicle or with 100 nM of MRS1220 or 3 μM of U0126 followed by a 3-hour incubation with 50 μg/ml of H1299-derived EVs. The relative expression levels of IL8, AREG, IL6, and VEGF was determined by quantitative real-time PCR as described under "Materials and Methods." Results are presented as percentage of maximal upregulation measured in the absence of inhibitor. Data are means ± SEM derived from three independent experiments. P values (U0126 vs. vehicle): IL8 .04, AREG .02, IL6 8.5E-4, VEGF 5.1E-5; P values (MRS1220 vs. vehicle): IL8 3E-3, AREG 1.3E-6, IL6 4.5E-4, VEGF 2E-3.

25% inhibition) (Figure 7). Collectively, these results indicate that the upregulation of tissue remodeling genes is driven by distinct signaling pathways that are elicited by autocrine signaling of the A3R as well as by other EV-activated pathways.

Discussion

The underlying mechanisms and functional consequences of MC activation in the TME remain by largely unresolved. Moreover, clinical evidence is split between data demonstrating antitumorigenic impact of MC infiltration into the TME and protumorigenic influence [6−9]. Therefore, there is a pressing need for deciphering the enigma of the cancer-MC cross talk. Towards this aim, we have undertaken to investigate the possibility that MCs are directly activated by cancer cells, which may shift their phenotype from immune protective into protumorigenic. Indeed, we were able to demonstrate that in co-cultures MCs form synapses with cancer cells and are directly activated by contact with the cancer cells [21]. However, because such a mechanism would only apply to intratumorally localized MCs, we have extended these studies to address the possibility of MC activation by EVs that are released by the cancer cells. We envisioned that should such a mechanism exist, then it could account for the cross talk between the tumor and MCs that are localized at peritumoral zones. These studies yielded a number of important observations: first, our results clearly demonstrate that EVs released by cancer cells can activate MCs, as indicated by the stimulated phosphorylation of the ERK1/2 MAP kinases, used here as a reporter for the MC activation status. Second, we show that MCs take up the cancer cell−derived EVs; however, activation of the ERK1/2 MAP kinases is rapid, preceding EV uptake. Therefore, we envision that EVs could drive distinct functions in

MCs. Contact of the MCs with EV membranes initiates signaling pathways, including the activation of PI3K(s) and ERK1/2 MAP kinases, which are partially contributed by autocrine signaling of adenosine. The latter depends on the activity of CD73 and is mediated by the adenosine A3 receptor. This adenosinergic arm of EV-stimulated MC activation partially contributes to the upregulation of tissue remodeling genes, including the angiogenic genes IL8, IL6, and VEGF and the growth factor AREG. In particular, upregulation of AREG, a ligand of the EGF receptor that plays an important role in the progression of lung cancer [32], is inhibited by 80% by MRS1220, the specific antagonist of the A3R. Our results also hint to the existence of a negative constraint, of yet an unknown nature, that is imposed on ERK1/2 signaling by protein kinase C and the p38 MAP kinase, whose inhibition potentiates ERK1/2 phosphorylation in EV-activated MCs. What benefit might the tumor gain in upregulating the expression of tissue remodeling genes in MCs is presently unknown. An interesting idea would be that these mRNAs are packaged and released in MC-derived EVs. Such a mechanism would implicate EVs in mediating a bidirectional MC-tumor cross talk, generating a vicious cycle of bidirectional reprogramming.

A second, slower arm of MC modulation is likely to result from the uptake of the EVs. EVs have been shown to contain proteins, including receptors, as well as mRNA and microRNA [33], which are likely to influence MC function following their uptake. Proteomic analyses revealed that EVs derived from NSCLC patients contain Rab GTPases and a variety of signaling molecules [34−36]. Therefore, NSCLC-derived EVs are likely to impact both intracellular trafficking and signaling events following their uptake. The relative contribution of signaling events triggered by contact with EVs or their uptake is presently unknown. Future studies will aim to distinguish between these mechanisms of MC activation that may either act in synergy or counteract each other, giving rise to pro- or antitumorigenic outcomes.

In conclusion, we demonstrate here for the first time direct activation of MCs by cancer cell−derived EVs, a mechanism that could account for the activation and reprogramming of MCs that reside in the tumor periphery in the TME. Furthermore, similarly to MC activation by contact with cancer cells [21], MC activation by cancer cell−derived EVs involves adenosine signaling that is mediated by the A3R. Therefore, at least one arm of cross talk between cancer cells and intratumoral or peritumoral MCs is shared. Given the well-established role of adenosine in cancer, by promoting tissue remodeling and angiogenesis and by conferring an immunosuppressive environment [37−41], our results further emphasize the important role played by MCs in the TME. Therefore, understanding how MCs are activated in the TME and deciphering the consequences of MC activation may not only untangle the controversy regarding the role of MCs in the TME but also pave the road to MC manipulation as part of the oncology regimens.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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