

New intriguing roles of ATP and its receptors in promoting tumor metastasis

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Introductory note

Despite the notion that platelets can promote tumor metastatization dates back to the 19th century [1], the underlying mechanisms have remained elusive. Two hypothesis have been proposed to explain platelet-facilitated tumor spreading: 1) a pro-angiogenic activity of platelet-derived cytokines, and 2) a protective effect of the platelet coat that shields tumor cells from both immune cell aggression and shear stress [1]. In this issue, Elena Adinolfi discusses two recently published papers, one in *Cancer Cell* and the second one in *Carcinogenesis*, which highlight the involvement of nucleotides in platelet-mediated transmigration of tumor cells through vascular endothelia (Highlight N. 1) and the central role of the P2X7 receptor in cancer metastatization (Highlight N. 2). Globally, these papers testify the continued interest in unfolding the involvement of the purinergic system in cancer dissemination and support the development of novel purinergic-based potent and effective drugs for the treatment of cancer.

Highlight N. 1

Schumacher D, Strilic B, Sivaraj KK, Wettschureck N, Offermanns S. Platelet-derived nucleotides promote tumor-cell transendothelial migration and metastasis via P2Y2 receptor. *Cancer Cell* 2013, 24,130-137.

Article summary

The study by Schumacher and colleagues [2] unveils a central role for nucleotides in platelet-mediated transmigration of tumor cells through vascular endothelia. With a series of elegant experiments the authors show that co-incubation of tumor cells with platelets triggers ATP release and facilitates tumor cell passage across endothelial layer. Platelet-induced extravasation of tumor cells was eased by tight junction relaxation, a process crucially dependent on ATP release, as it was completely lost upon treatment with apyrase, a powerful nucleotide degrading enzyme.

The authors then investigated the role of purinergic signaling in metastatic spreading in Munc 13–4 deficient mice. These mice, which are genetically-deleted of the exocytosis priming protein Munc 13–4, are deficient in the exocytosis of dense granules, and therefore are unable to release ATP and ADP through this pathway. B16 melanoma or LLC1 Lewis lung carcinoma tumors were induced in Munc 13–4 deficient and wt mice. Very interestingly, primary tumor mass formation did not differ in the two mouse strains, while metastatic spreading turned out to be strongly reduced in the Munc 13–4 deleted animals.

In subsequent experiments the authors set to identify the endothelial nucleotide receptor involved in platelet-induced tumor cell extravasation. The sole P2 receptors expressed by the endothelia were P2Y1, P2Y2 and P2X4. Pharmacological blockade or genetic ablation of P2Y1 and P2X4 did not affect platelet-dependent, tumor cell transmigration. On the other hand, P2Y2 deletion dramatically reduced platelet-dependent endothelial permeability increases, loosening of inter-endothelial junctions, and tumor cell extravasation. Metastatic spreading was also consistently reduced in P2Y2 KO mice. In the absence of reliable selective pharmacological P2Y2 antagonist, the authors down-modulated endothelial P2Y2 by RNA silencing. This procedure effectively reduced tumor cells trans-endothelial migration. On the contrary, P2Y2 stimulation with

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ATP or ATP_γS facilitated tumor cells migration across the vessel cells.

Finally, data from Munc 13–4 or P2Y2 deficient mice were corroborated by experiments of bone marrow transplantation in irradiated mice. Implant of bone marrow from either Munc 13–4 or P2Y2 KO mice into wt animals reduced metastatic dissemination, thus showing that hematopoietic cells are the key determinants of the metastatic phenotype. Consistent with the notion that P2Y2 and dense granules secretion are on the same pathway, bone marrow chimeras of Munc 13–4 and P2Y2 KO mice did not show an additional reduction in tumor dissemination. Of particular interest is the *in vivo* observation that in lungs the endothelial barrier is permeable to tumor cells, and that platelet-derived nucleotides and P2Y2 receptor activation promote tumor cell extravasation also in this context.

Commentary

The crucial role of platelet secreted nucleotides in blood coagulation is well known and has been exploited for the production of several antithrombotic drugs targeting platelets P2Y12 receptor such as clopidogrel, pasugrel and ticagrelor [3]. Moreover, it is also well established that platelets facilitate tumor cell survival in the blood. In the present paper, Schumacher and colleagues provide an elegant demonstration of the role played by dense granule exocytosis and purinergic signalling in tumor metastasis, and identify endothelial P2Y2 as the main P2 receptor involved. Needless to say, this points to P2Y2 as an attractive target to develop metastasis preventing therapies without affecting hemostasis.

An issue that was not addressed by this study is if and how tumor cells stimulate ATP release from platelets. Is this mediated by cell-cell interaction or via secretion of yet-to-be identified extracellular messengers? Is the tumor cell mimicking the conditions required for clot formation? These are open questions that may be worth of an depth investigation.

Interestingly, the authors show that it is ATP per se, and not platelets, which is required to facilitate tumor cell extravasation. This evidence on one hand supports the view that an anti-metastatic therapy based on P2Y2 inhibition might not affect hemostasis; on the other hand it also suggests that ATP release from sources other than platelets would similarly ease tumor cell migration across endothelia. This is of particular interest in view of the demonstration that tumor microenvironment is rich in extracellular ATP [4].

Highlight N. 2

Jelassi B, Anchelin M, Chamouton J, Cayuela ML, Clarysse L, Li J, Gore J, Jiang LH, Roger S. Anthraquinone emodin inhibits MDA-MB-435 s human cancer cell invasiveness by antagonizing P2X7 receptors. *Carcinogenesis* 2013; 34(7):1487–96.

Article summary

In this study Jelassi [5] and colleagues demonstrated a role for P2X7 in mediating breast cancer cell invasiveness and the efficacy of its antagonist emodin in preventing tumor dissemination. Their results further support and integrate the findings of two other recent studies showing respectively that P2X7 receptor affects extracellular matrix invasion through cathepsin secretion [6] and that emodin acts as P2X7 antagonist in macrophages [7].

In the present paper Jelassi et al. investigated the expression of P2X and P2Y receptors in the following cell lines, previously characterized for their invasive potential: MF-10A non-cancerous and MDA-MB468 breast cancer cells that both show a limited spreading capacity, plus MDA-MB-435 s breast and A549 lung cancer lines that on the contrary are prone to metastasize. Among the several P2 receptors present in these cells lines, only P2X7 expression was related to invasive potential as MF10A and MDA-MB468 cells were P2X7 negative, while MDA-MB435s and A549 expressed the receptor.

Emodin has been long used in traditional Chinese medicine as an antiinflammatory and tumor suppressive drug. Therefore the authors tried to elucidate if part of the anti-tumoral effect of this substance could be due to P2X7 inhibition.

In a first set of experiments, Jelassi et al. demonstrated that, in MDA-MB-435 s cancer cells, 10 μM emodin was able to reduce both ATP mediated intracellular calcium rise and evoked currents. To test the selectivity of emodin on different human P2X receptors, authors recorded ATP evoked currents in the presence of the compound on HEK293 cells transfected with all human P2X receptors. While incubation of P2X7 positive HEK293 cells with emodin caused a maximal current inhibition of approximately 70 %, with an IC₅₀ of 3 μM, no other human P2X receptor was blocked by the compound. Unexpectedly, emodin was able to increase P2X2 dependent ATP-evoked currents by reducing receptor's desensitization.

In a second set of experiments, Jelassi and colleagues tested the effect of emodin on metastatic potential of breast cancer cells.

The ability of tumor cells to degrade the extracellular matrix and either migrate away for primary tumor site or invade different tissues and organs is central in metastatization. These characteristics of cells are generally tested *in vitro* by measuring their power to lyse and cross experimental matrixes such as agarose, gelatin or Matrigel. Activation of P2X7 receptor by treatment with 3 mM ATP was able to increase the gelatinolytic activity and Matrigel transmigration of MDA-MB435s and A549 cells. Both phenomena were significantly reduced in the presence of 1 μM emodin as well as the synthetic P2X7 antagonist A438079 (10 μM). Emodin showed no similar effect either on P2X7 receptor silenced MDA-MB435s or in P2X7 negative MDA-MB468 cells.

In accordance to what shown by previous assays, P2X7 stimulation conferred an infiltrating appearance to MDA-MB435s cells causing their elongation. This neurite like shape was lost upon co-treatment with ATP and emodin.

In vitro data were further supported by in vivo experiments carried out in the zebra fish model of micrometastasis. This assay is based on injection of labeled cancer cells in zebra fish embryos and detection of cells migrated outside the yolk sac, which are assimilated to metastatic formations. Pretreatment of MDA-MB435s cells with either 1 or 10 μ M emodin resulted in a reduction of their migration respectively of the 41 % and 47 % of while had no effect on MDA-MB468.

Commentary

The idea that P2X7 receptor could support oncogenesis [8–10] has recently gained strength thanks to a series of papers implicating the receptor in in vivo tumor growth, neo-vascularization and metastatization [6, 11, 12]. The study from Jelassi and colleagues [5] further support these findings showing the efficacy of emodin, a Chinese traditional medicine compound, in reducing P2X7 mediated malignant progression. Emodin is a naturally occurring anthraquinone derivative, isolated from roots and barks of numerous plants, molds and lichens. Such as other herbal compounds, emodin has been used for ages showing healing effects whose molecular mechanism is largely unknown [13]. Jelassi et al. convincingly show specific activity of emodin on P2X7 receptor expressed by invasive breast cancer cells. Together with inhibitory effect on ATP evoked currents and calcium permeabilization [7], emodin also significantly reduced P2X7 mediated cancer cells dissemination. Data showing emodin dependent reduction of tumor cells gelatinolytic activity are in accordance with previous studies reporting an increased infiltration of soft agar by HEK293 cells expressing P2X7 A and B isoforms [14]. Similarly, evidence on the effect of emodin on Matrigel infiltration and in the zebrafish model of metastatization follow previous reports showing an equivalent impact of other P2X7 blockers [6]. Effect of emodin, and other P2X7 antagonists, on animal models of metastatization closer to human, such as mouse, remains to be tested.

The study from Jelassi et al. offers a new explanation for the antitumoral effect of emodin inferring that it could be related to P2X7 dependent tumor cell metastatization. However, like emodin [13] also other P2X7 antagonists hamper tumor growth, glycolysis and neoplastic vascularization [11, 15]. Indeed, Jelassi et al. did not exclude emodin-dependent reduction of breast cancer cell viability. So it is tempting to speculate that emodin, acting at P2X7 receptor, would exert an anti-cancer activity by inhibiting several pathways including proliferation, vessel formation and metastatization. Moreover, activity of emodin on other P2X receptors, such as P2X2 [16] also requires to be further investigated.

Summary and future perspectives

The final and often lethal step of tumoral evolution is metastatization. This phenomenon is not only driven by intrinsic alterations of malignant cells but also by interactions with cells and molecules present in the peri-tumoral space. Steps involved in metastatization include extracellular matrix degradation and invasion, extravasation in the blood and lymphatic vessels leading to distant site invasion. The studies described in this article [2, 5] strongly suggest that ATP is a crucial microenvironmental element in driving metastatic spread. While Jelassi et al. identified ATP as a mediator of infiltration, Schumacher et al. pinpointed the nucleotide as endothelia relaxant, facilitating tumor cell escape. Reported evidence strongly suggests that targeting purinergic receptors in cancer will prove beneficial in reducing metastatic dissemination. This data, together with the proposed detrimental effect of P2X7 antagonists on tumoral proliferation and neo-vascularization [11], support the hypothesis that the study of purinergic signaling will soon offer potent and effective drugs for the treatment of cancer.

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Elena Adinolfi, Ph.D. is an assistant Professor of Clinical Pathology at the University of Ferrara, Italy. She has been working on purinergic signalling for the last 15 years focusing her attention on extracellular ATP and P2X7 receptor. She has been establishing a link amongst P2X7 activity increased cell metabolism and tumoral transformation. At the moment she is investigating the oncogenic role of P2X7 in live models of carcinogenesis.