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Cancer-derived extracellular vesicles: friend and foe of tumour immunosurveillance

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Extracellular vesicles (EVs) are important players of intercellular signalling mechanisms, including communication with and among immune cells. EVs can affect the surrounding tissue as well as peripheral cells. Recently, EVs have been identified to be involved in the aetiology of several diseases, including cancer. Tumour cell-released EVs or exosomes have been shown to promote a tumour-supporting environment in non-malignant tissue and, thus, benefit metastasis. The underlying mechanisms are numerous: loss of antigen expression, direct suppression of immune effector cells, exchange of nucleic acids, alteration of the recipient cells' transcription and direct suppression of immune cells. Consequently, tumour cells can subvert the host's immune detection as well as suppress the immune system. On the contrary, recent studies reported the existence of EVs able to activate immune cells, thus promoting the tumour-directed immune response. In this article, the immunosuppressive capabilities of EVs, on the one hand, and their potential use in immunoactivation and therapeutic potential, on the other hand, are discussed.

This article is part of the discussion meeting issue 'Extracellular vesicles and the tumour microenvironment'.

1. Introduction

Extracellular vesicles (EVs) were initially described a few decades ago; all cells release certain membrane vesicles with a great variety of important functions. In 1984, vesicle release was described as a novel mechanism of transferrin receptor secretion in sheep reticulocytes [1]. This release is linked to the formation of intracellular exosomes, originating from an endosomal multi-vesicular body (MVB), which fuse with the cells plasma membrane [2].

Originally, budding of vesicles from the plasma membrane was suggested to be part of the lysosomal degradation pathway, responsible for the excretion of cell debris [3] and emergency membrane repair [4]. Subsequent studies drew attention to the role of B lymphocyte-secreted EVs in regulation of the immune response [5] and, about a decade later, intercellular exchange of mRNAs and miRNAs via EVs was confirmed by Valadi *et al*. [6].

Cells produce and release different types of EVs, which can be distinguished according to their size: apoptotic bodies (1000–5000 nm) characterize the largest fraction, microvesicles (200–1000 nm) comprise the intermediate fraction and exosomes (30–150 nm) are the smallest fraction [7]. Exosomes are ubiquitously released by all cells, including malignant cells, and are present in the body fluids [8]. In contrast with other EVs, the biogenesis of exosomes starts with an invagination of the plasma membrane. During maturation, the initial endosome experiences several inward invaginations forming numerous

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intraluminal vesicles and thus incorporating components of the cytosol. The endosome becomes a so-called MVB comprising multiple vesicles which contain different proteins and nucleic acids [9]. MVBs can subsequently fuse with the plasma membrane releasing the contained exosomes into the extracellular space. Apart from that, MVBs can enter the lysosomal degradation pathway. The fate of the MVBs is dependent on the amount of ceramides contained in the membrane-associated lipids [7]. The exosomes released in this manner carry a characteristic and cell type-specific composition of nucleic acids, proteins, enzymes, lipids, cytokines and other soluble factors inherited from the parental cell [10,11]. The endosomal sorting complex required for transport (ESCRT) is responsible for packing and trafficking of exosomes or subtypes of exosomes [12]. During this process, exosomes are loaded with components of the ESCRT and associated molecules [13,14], which are common markers used to identify exosomes of endocytic origin [7,11]. These molecules include parent cell-characteristic annexins, flotillin, GTPases, lipids and cholesterol [15–17], as well as tetraspanins (CD9, CD63, CD81, CD82) [18,19] and proteins of the accessory ESCRT pathway (e.g. ALIX and TSG101) [13]. Although the content of the exosomes does not completely resemble the profile of the parental cell, the partial similarity inspired the idea of using exosomes as biomarkers for tumours. Differences in the profile of parent cells and exosomes indicate the participation of still unknown processes [20,21]. Besides the ESCRT, other sorting mechanisms dependent on raft-based microdomains have been proposed to be involved in the genesis of exosomes [22,23]. Apart from the exosome fraction, certain microvesicles, the so-called ectosomes, can be formed by membrane blebbing [9]. These EVs are also suggested to play a role in intercellular communication. Yet the differentiation between exosomes and microvesicles is not completely understood [9]. This challenges the use of vesicle size as reliable indicator for the definition of EVs and both fractions need to be analysed to identify suitable EV-associated biomarkers [20].

Composition, biogenesis and secretion of EVs/exosomes are adaptive processes influenced by extrinsic stimuli including cellular stress. Cells are able to respond to intracellular stress situations by secretion of vesicles influencing their environment [24]. Moreover, they play an important role in the host's immune response. Among others, dendritic cell-derived exosomes (Dex) are involved in the immune system's response to tumours and promote the proliferation and cytolytic activity of natural killer (NK) cells [25]. Malignant cells are frequently challenged with stress situations such as hypoxia, starvation or chemotherapeutic drugs in their microenvironment which they need to overcome to facilitate progression of the tumour [24]. It is well known that tumours shape their microenvironment by EV/exosome-mediated communication with the surrounding stromal tissue, thus promoting proliferation, angiogenesis, suppression of the host's immune defence and initiation of pre-metastatic niches [26]. Further, the release of tumour-derived EVs/exosomes (T-EVs) is frequently increased in tumour patients [27] and especially elevated after chemotherapy or photodynamic treatment [28]. Interestingly, the tumour suppressor p53, which is tightly connected to the aetiology of cancer, is involved in the regulation of vesicle release [29]. Protein microarray analysis of peripheral blood mononuclear cells (PBMCs) revealed an immunosuppressive effect of T-EVs at high concentrations, whereas PBMCs showed an activated phenotype at low concentrations [30]. In line with the latter, T-EVs can also carry so-called tumour-associated antigens (TAAs), costimulatory molecules and major histocompatibility complexes (MHC) components mediating a stimulatory effect on immune cells [31,32]. These findings suggest a switch in the virtue of EVs from immunoactivation towards immunosuppression during tumour progression. To date, the underlying molecular basis for this functional alteration remains largely elusive.

2. Immunosuppression by cancer-derived extracellular vesicles

T-EV-mediated communication is likely to benefit the tumour's progression and survival. During their progression, tumours develop several T-EV-based approaches to interfere with the host's immune system counteracting anti-tumour activities. This requires some sort of interaction between T-EVs and immune cells such as binding or internalization of the vesicles [33]. Ligands or antigens present in or on T-EVs can directly interact with receptors on lymphocytes or bind to cellular MHC receptors, respectively. Receptor-mediated uptake allows T-EVs to fuse with the cell's plasma membrane and release their contents into the cytoplasm. In addition, phagocytic cells (e.g. macrophages and dendritic cells; DCs) can easily internalize T-EVs. T-EVs interacting with surface molecules on T-cells transfer signals and by this alter their function [34]. To bypass the host's immune response, tumours subvert the recognition by cytotoxic T-lymphocytes (CTLs), impair the antigen presentation by antigen-presenting cells or interfere with the host's immune response. Moreover, immune cells can be tricked to support the tumour. In these strategies, the appropriate surface proteins, intravesicular cytokines or nucleic acids, with which EVs are equipped, play a crucial role [35].

T-EVs containing so-called death ligands, e.g. Fas ligands or tumour necrosis factor-α (TNF-α), hold the potential to directly induce cell death in immune cells through activation of the death receptor family members TNF receptor 1 (TNF-R1) and Fas receptor (FasR), respectively. Activation of these receptors is tightly linked to the induction of necrosis and caspase-dependent cell death [36–40].

One strategy of immune evasion is direct EV-mediated immune suppression. The primary target of this strategy are the CTLs. The potential of T-EVs to inhibit the growth of $CD8^+$ CTLs is reported for several cancer types [38,41]. Transforming growth factor-β (TGF-β) is one of the most prominent immunosuppressive cytokines found on the surface of EVs. Suppression of NK cell function and T-cell proliferation by vesicular TGF-β on T-EVs was observed in patients suffering from acute myeloid leukaemia [27] and breast cancer [42]. Peinado *et al*. [43] demonstrated the potential of T-EVs derived from highly metastatic melanomas to reprogramme bone marrow cells to form a melanoma-friendly environment. Thus, T-EVs are able to interfere with the development and differentiation of haematopoietic cells as well as with the functions of mature cells [44,45]. Additional to direct suppression and cell death induction, T-EVs can induce the differentiation of regulatory T cells (Tregs) and myeloid-derived suppressor cells [38,46].

Host cells express MHC-I molecules, protecting them from the attack of CTLs, whereas tumour cells expressing

MHC-I/TAA complexes are destroyed by CTLs. Downregulation of the MHC-I/TAA complexes allow the tumour to escape detection by the adaptive immune system [47]. However, cells lacking the MHC-I complex are approached and eliminated by NK cells [48]. To avoid the attack of NK cells, tumours are able to release EVs influencing the cytotoxic activity of NK cells [49], which is regulated by an equilibrium of activating and inhibitory receptors. The ligands of the NK cell-activating receptor NK group 2, member D (NKG2D) MHC class I chain-related proteins A and B (MIC-A and MIC-B) and UL-16-binding protein [50] are present on the surface of EVs [51]. EVs bearing NKG2D ligands act as bait for NK cells by distracting the immune cells from the tumour [51]. Additionally, these EVs elicit a downregulation of NKG2D receptors on NK cells [52,53]. Owing to the high proliferation rate of many tumour cells, the tumour is likely to outgrow the blood supply, resulting in large parts of the tumour tissue being supplied with low oxygen concentrations. In order to survive in the hypoxic microenvironment, tumour cells are known to adapt their metabolism [54]. A study published by Berchem *et al*. proved that T-EVs emerging from hypoxic conditions had a stronger inhibitory impact on NK cells compared to T-EVs originating from normoxic conditions. The increased immunosuppressive potential was attributable to the transfer of miR-23a and TGF-β to NK cells [55]. In addition, increased levels of miR-4498 were observed in hypoxic T-EVs derived from melanoma cells [35]. CD83, which is a key in the communication between cells of the innate and adaptive immune response, is regulated by miR-4498 [56].

In vitro studies indicate the intercellular exchange of nucleic acids via EVs [6,57]. Ding *et al*. demonstrated an increase in cancer-related miRNAs as well as inhibition of a wealth of mRNAs in DCs exposed to pancreatic cancer-derived T-EVs. Interestingly, the authors revealed an inhibition of the MHC II transcription factor regulatory factor X-associated protein (RFXAP) by miR-212-3p received from T-EVs. This was further confirmed by clinical data negatively correlating miR-212-3p and RFXAP in pancreatic cancer tissue [58]. The presence of inhibitory miRNAs or mRNAs promoting the aetiology of cancer and negatively influencing the host's immune response was also suggested for T-EVs derived from other cancer species [59,60]. A recent study stated that T-EV-recipient cells experience a regulation of genes responsible for the immune response [61]. In detail, gene profiles of several human T-cell subsets exposed to T-EVs *in vitro* were analysed. Tregs were most sensitive to EV-mediated effects and experienced downregulation of genes involved in the adenosine pathway, which induces a high expression of CD39 and enhanced adenosine production [61]. Extracellular ectonucleotidases such as CD39 contribute to high levels of the purine nucleoside adenosine [62], which is a powerful immune regulator attenuating local immune responses [63]. Besides, T-EVs caused an upregulation of inhibitory genes in $CD4^+$ T cells that are responsible for loss of function via downregulation of CD69 expression. T-EVs carrying the ectonucleosidases CD73 and CD39 on their surface can, moreover, produce extracellular adenosine, directly interfering with T cells [64]. Concomitant with induction of necrosis, TNF-containing T-EVs from melanoma cells induce the production of intercellular reactive oxygen species in T-cells, which impairs the T-cell receptor signalling pathway and hence leads to a decrease in T cells [65].

3. Extracellular vesicle-mediated immunoactivation

Apart from the critical immunosuppressive potential of T-EVs, vesicles bearing immune-activating effects have been described recently. This mirrors the diverse and differentiated functions of EVs. Latest research has focused on the immunostimulatory properties of dendritic cell-derived exosomes (Dex) and their potential value for immunotherapy [66,67]. Dex maintain the central functions of DCs: presentation of TAAs and activation of TAA-specific immune responses. Their membrane harbours a variety of molecules responsible for antigen presentation (MHC class I, class II, CD1), adhesion (intercellular adhesion molecules), costimulatory signals (CD86, CD40) and docking (integrins) [68,69].

Viaud *et al*. demonstrated that Dex promote an interleukin-15 Rα- (IL-15Rα) and NKG2D-dependent proliferation and activation of NK cells in a murine *in vivo* model, resulting in an anti-metastatic effect. Furthermore, human Dex are equipped with NK cell-activating NKG2D ligands. A Dex-based vaccine was able to restore NKG2D-dependent functions of NK cells in half of the tested melanoma patients [25].

The melanoma-associated tumour antigens (MAGEs) are usually not present on host cells but are commonly expressed by different tumour species [70]. An early phase I clinical study addressing the therapeutic use of MAGE antigen-loaded Dex in 15 $MAGE3^+$ advanced melanoma patients reported a response in one patient, one minor response and two stabilizations of disease. Although almost two-thirds of patients showed NK cell effector functions, no MAGE-specific T-cell responses were observed in the peripheral blood [71]. In a second phase I clinical trial performed by another group, one-third of advanced $MAGE^+$ non-small cell lung cancer (NSCLC) patients developed MAGE3.A1-specific systemic immune responses in line with upregulation of NK cell activity [72]. TAA-loaded Dex have proven their feasibility of large-scale production and outstanding safety profile in these studies [71,72]. In contrast with Dex from immature DCs, new approaches using EVs derived from TLR4 L- or interferon (IFN)-γ-maturated DCs showed improved Dex-induced T-cell stimulation [73–76]. A recent phase II clinical trial applying IFN-γ-Dex loaded with MHC class I- and class II-restricted cancer antigens as immunotherapy of NSCLC patients after chemotherapy showed that the expression of MHC class II on Dex correlated with the expression of the NK cell activating NKp30 ligand BCL2-associated athanogene 6 (BAG6) [77]. The chaperone BAG6 plays a role in a multitude of cellular processes and was identified as ligand of the activating NK cell receptor NKp30 [78,79]. The expression patterns of both BAG6 and MHC-II are tightly connected and controlled by the IFN-γ-inducible class II transactivator (CIITA) [80]. BAG6 is necessary for the accumulation of HSP70 [81,82], which bears the potential to activate the immune response. HSP70 induces the maturation of DCs and promotes the phagocytosis of tumour cells as well as cross-presentation of chaperoned peptides. NK cells are required for the interaction of DCs and HSP70 to induce a CTL response and anti-metastatic effect *in vivo* [83]. Moreover, HSP70/BAG4 surface-positive T-EVs specifically facilitate migration and HSP70 reactivity of NK cells. HSP70-specific antibodies can inhibit the T-EV-induced cytolytic activity of NK cells [84]. It should be noted that a soluble form of BAG6 (sBAG6) in the plasma of chronic lymphocytic

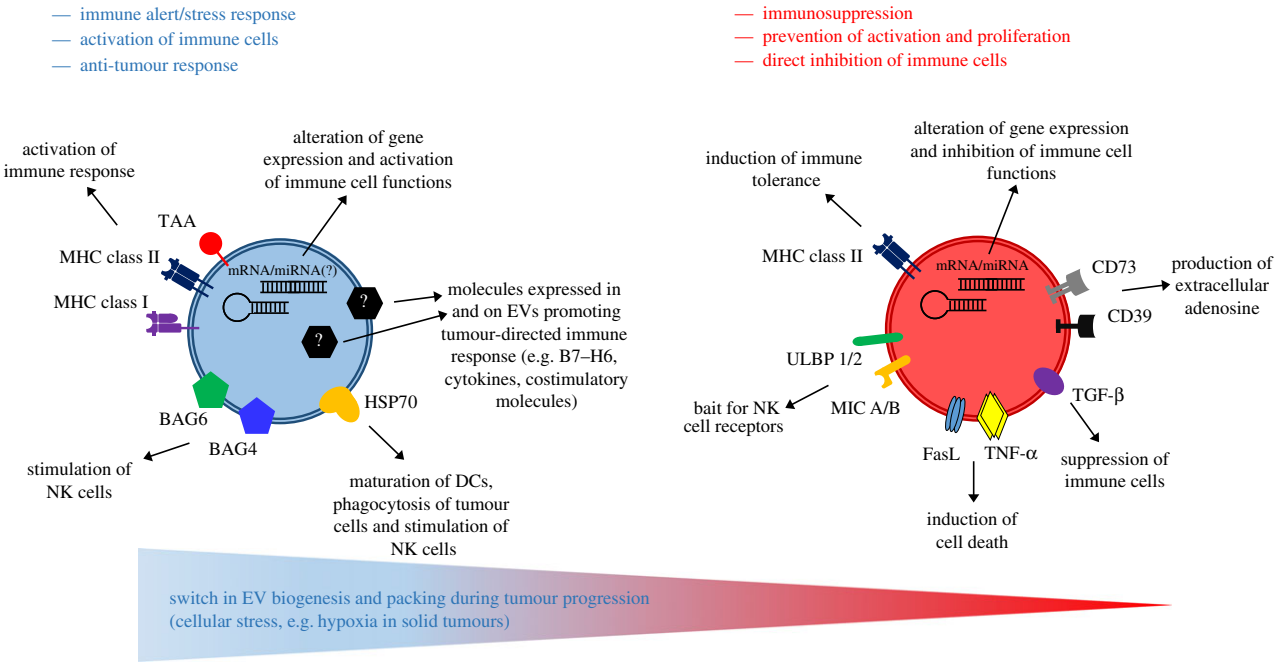

Figure 1. Scheme of EVs carrying immunoactivating and immunosuppressing molecules and their impact on the immune response. Future research identifying yet unknown molecules expressed in and on the surface of EVs might clarify the mechanism underlying the switch from immune altering EVs to immunosuppressing T-EVs during tumour progression. Moreover, new biomarkers for tumours and therapeutic approaches enhancing the host's tumour-directed immune response could be envisioned.

leukaemia patients critically impaired the function of NK cells [85], whereas vesicular BAG6 is a powerful activator of NK cells [86]. According to Besse *et al*. [77], a possible explanation for the opposing virtue of EV-BAG6 and sBAG might be the interplay of BAG6 and HSP70 on exosomes to activate NK cells via co-engagement of NKp30 and a second regulatory NK cell receptor, CD94, also known as NKG2. An upregulation of CD94 receptors on NK cells is described to correlate with enhanced cytolytic activity after stimulation with HSP70 or HSP70 and IL-2 [87,88]. Alternatively, the oligomerization of EV-BAG6 was discussed as possible explanation for the contrasting activities of EV-BAG6 and sBAG6 [79]. The cytosolic immune-sensing receptor retinoic acid-inducible gene I (RIG-I) is ubiquitously expressed in nucleated cells, including malignant cells [89], and activated by viral 5′-triphosphorylated RNA [90,91]. Dassler-Plenker *et al*. discovered a novel mechanism of RIG-I-mediated release of EVs with anti-tumour activity from tumour cells. The EVs showed increased expression of BAG6 on their membrane, thus activating NKp30-mediated cytotoxic activity of NK cells [92].

Besides BAG6, B7-H6, which is a member of the B7 family of immunoreceptors, is a well-known cell surface ligand for the NK cell-activating receptor NKp30 [93,94]. In contrast to BAG6, the expression of B7-H6 is restricted to tumour cells [93,95]. B7 family members are induced on myeloid cells upon inflammatory stimuli [96,97], but the underlying mechanism remains unresolved. Matta *et al*. discovered B7-H6 in the vesicle fraction after ultracentrifugation, indicating that B7-H6 could be included in EVs present in patients' serum or be present as soluble variant. *In vitro* experiments revealed that the isolated B7-H6 originated from activated monocytes and neutrophils and possessed the potential to modulate the activity of NK cells [98].

4. Conclusion and future challenges

Tumour cell-derived EVs may either trigger or, on the contrary, suppress anti-tumour immune responses and their biological role is controversial (figure 1). Some molecules expressed on immune-activating or suppressive EVs are indicated; however, the plasticity of T-EVs or differences in EV subtypes remain to be investigated. This analysis will enable us to identify the cargo (including nucleic acids, lipids and proteins) which is responsible for the functional activity of EVs or of a given EV subtype. There is emerging evidence that DNA damage or stimuli of the microenvironment such as hypoxia or receptor activation impact on EV biosynthesis, cargo loading or their release. A better molecular understanding of the downstream pathways directing EV composition and secretion is mandatory for the rational therapeutic application of EVs to combat cancer.

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References

1. Pan BT, Johnstone R. 1984 Selective externalization of the transferrin receptor by sheep reticulocytes *in vitro*. Response to ligands and inhibitors of endocytosis. *J. Biol. Chem.* **259**, 9776–9782.
2. Pan BT, Teng K, Wu C, Adam M, Johnstone RM. 1985 Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *J. Cell Biol.* **101**, 942–948. (doi:10.1083/jcb.101.3.942)
3. Rodríguez A, Webster P, Ortego J, Andrews NW. 1997 Lysosomes behave as Ca^{2+}-regulated exocytic

vesicles in fibroblasts and epithelial cells. *J. Cell Biol.* **137**, 93–104. (doi:10.1083/jcb.137.1.93)

4. McNeil PL, Kirchhausen T. 2005 An emergency response team for membrane repair. *Nat. Rev. Mol. Cell Biol.* **6**, 499–505. (doi:10.1038/nrm1665)
5. Raposo G *et al.* 1996 B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med.* **183**, 1161–1172. (doi:10.1084/jem.183.3.1161)
6. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. 2007 Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* **9**, 654–659. (doi:10.1038/ncb1596)
7. Brinton LT, Sloane HS, Kester M, Kelly KA. 2015 Formation and role of exosomes in cancer. *Cell. Mol. Life Sci.* **72**, 659–671. (doi:10.1007/s00018-014-1764-3)
8. Keller S, Ridinger J, Rupp A-K, Janssen JWG, Altevogt P. 2011 Body fluid derived exosomes as a novel template for clinical diagnostics. *J. Transl. Med.* **9**, 86. (doi:10.1186/1479-5876-9-86)
9. Cocucci E, Meldolesi J. 2015 Ectosomes and exosomes: shedding the confusion between extracellular vesicles. *Trends Cell Biol.* **25**, 364–372. (doi:10.1016/j.tcb.2015.01.004)
10. van der Pol E, Boing AN, Harrison P, Sturk A, Nieuwland R. 2012 Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacol. Rev.* **64**, 676–705. (doi:10.1124/pr.112.005983)
11. Raposo G, Stoorvogel W. 2013 Extracellular vesicles: exosomes, microvesicles, and friends. *J. Cell Biol.* **200**, 373–383. (doi:10.1083/jcb.201211138)
12. Cocucci E, Racchetti G, Meldolesi J. 2009 Shedding microvesicles: artefacts no more. *Trends Cell Biol.* **19**, 43–51. (doi:10.1016/j.tcb.2008.11.003)
13. Colombo M *et al.* 2013 Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *J. Cell Sci.* **126**, 5553–5565. (doi:10.1242/jcs.128868)
14. Kowal J, Tkach M, Thery C. 2014 Biogenesis and secretion of exosomes. *Curr. Opin. Cell Biol.* **29**, 116–125. (doi:10.1016/j.ceb.2014.05.004)
15. Wubbolts R, Leckie RS, Veenhuizen PTM, Schwarzmann G, Mobius W, Hoernschemeyer J. 2003 Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. *J. Biol. Chem.* **278**, 10 963–10 972. (doi:10.1074/jbc.M207550200)
16. Record M, Subra C, Silvente-Poirot S, Poirot M. 2011 Exosomes as intercellular signalosomes and pharmacological effectors. *Biochem. Pharmacol.* **81**, 1171–1182. (doi:10.1016/j.bcp.2011.02.011)
17. Subra C *et al.* 2010 Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. *J. Lipid Res.* **51**, 2105–2120. (doi:10.1194/jlr.M003657)
18. Rana S, Zoller M. 2011 Exosome target cell selection and the importance of exosomal tetraspanins: a hypothesis. *Biochem. Soc. Trans.* **39**, 559–562. (doi:10.1042/BST0390559)
19. Andreu Z, Yanez-Mo M. 2014 Tetraspanins in extracellular vesicle formation and function. *Front. Immunol.* **5**, 442. (doi:10.3389/fimmu.2014.00442)
20. Whiteside TL. 2016 Tumor-derived exosomes and their role in cancer progression. *Adv. Clin. Chem.* **74**, 103–141. (doi:10.1016/bs.acc.2015.12.005)
21. Villarroya-Beltri C, Baixauli F, Gutierrez-Vazquez C, Sanchez-Madrid F, Mittelbrunn M. 2014 Sorting it out: regulation of exosome loading. *Semin. Cancer Biol.* **28**, 3–13. (doi:10.1016/j.semcancer.2014.04.009)
22. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F. 2008 Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* **319**, 1244–1247. (doi:10.1126/science.1153124)
23. Gulbins E, Kolesnick R. 2003 Raft ceramide in molecular medicine. *Oncogene* **22**, 7070–7077. (doi:10.1038/sj.onc.1207146)
24. Jong OG, Verhaar MC, Chen Y, Vader P, Gremmels H, Posthuma G, Schiffelers RM, Gucek M, van Balkom BW. 2012 Cellular stress conditions are reflected in the protein and RNA content of endothelial cell-derived exosomes. *J. Extracell. Vesicles* **1**. (doi:10.3402/jev.v1i0.18396)
25. Viaud S *et al.* 2009 Dendritic cell-derived exosomes promote natural killer cell activation and proliferation: a role for NKG2D ligands and IL-15Ralpha. *PLoS ONE* **4**, e4942. (doi:10.1371/journal.pone.0004942)
26. Miller IV, Grunewald TGP. 2015 Tumour-derived exosomes: tiny envelopes for big stories. *Biol. Cell* **107**, 287–305. (doi:10.1111/boc.201400095)
27. Szczepanski MJ, Szajnik M, Welsh A, Whiteside TL, Boyiadzis M. 2011 Blast-derived microvesicles in sera from patients with acute myeloid leukemia suppress natural killer cell function via membrane-associated transforming growth factor-beta1. *Haematologica* **96**, 1302–1309. (doi:10.3324/haematol.2010.039743)
28. Aubertin K, Silva AKA, Luciani N, Espinosa A, Djemat A, Charue D, Gallet F, Blanc-Brude O, Wilhelm C. 2016 Massive release of extracellular vesicles from cancer cells after photodynamic treatment or chemotherapy. *Sci. Rep.* **6**, 35376. (doi:10.1038/srep35376)
29. Yu X, Harris SL, Levine AJ. 2006 The regulation of exosome secretion: a novel function of the p53 protein. *Cancer Res.* **66**, 4795–4801. (doi:10.1158/0008-5472.CAN-05-4579)
30. Hellwinkel JE, Redzic JS, Harland TA, Gunaydin D, Anchordoquy TJ, Graner MW. 2016 Glioma-derived extracellular vesicles selectively suppress immune responses. *Neuro Oncol.* **18**, 497–506. (doi:10.1093/neuonc/nov170)
31. Qazi KR, Torregrosa Paredes P, Dahlberg B, Grunewald J, Eklund A, Gabrielsson S. 2010 Proinflammatory exosomes in bronchoalveolar lavage fluid of patients with sarcoidosis. *Thorax* **65**, 1016–1024. (doi:10.1136/thx.2009.132027)
32. Altevogt P, Bretz NP, Ridinger J, Utikal J, Umansky V. 2014 Novel insights into exosome-induced, tumor-associated inflammation and immunomodulation. *Semin. Cancer Biol.* **28**, 51–57. (doi:10.1016/j.semcancer.2014.04.008)
33. Mulcahy LA, Pink RC, Carter DRF. 2014 Routes and mechanisms of extracellular vesicle uptake. *J. Extracell. Vesicles* **3**, 24641. (doi:10.3402/jev.v3.24641)
34. Zhang X, Yuan X, Shi H, Wu L, Qian H, Xu W. 2015 Exosomes in cancer: small particle, big player. *J. Hematol. Oncol.* **8**, 83. (doi:10.1186/s13045-015-0181-x)
35. Czernek L, Duchler M. 2017 Functions of cancer-derived extracellular vesicles in immunosuppression. *Arch. Immunol. Ther. Exp.* **65**, 311–323. (doi:10.1007/s00005-016-0453-3)
36. Walczak H. 2013 Death receptor-ligand systems in cancer, cell death, and inflammation. *Cold Spring. Harb. Perspect. Biol.* **5**, a008698. (doi:10.1101/cshperspect.a008698)
37. Taylor DD, Gercel-Taylor C, Lyons KS, Stanson J, Whiteside TL. 2003 T-cell apoptosis and suppression of T-cell receptor/CD3-zeta by Fas ligand-containing membrane vesicles shed from ovarian tumors. *Clin. Cancer Res.* **9**, 5113–5119.
38. Wieckowski EU, Visus C, Szajnik M, Szczepanski MJ, Storkus WJ, Whiteside TL. 2009 Tumor-derived microvesicles promote regulatory T cell expansion and induce apoptosis in tumor-reactive activated CD8+ T lymphocytes. *J. Immunol.* **183**, 3720–3730. (doi:10.4049/jimmunol.0900970)
39. Andreola G *et al.* 2002 Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. *J. Exp. Med.* **195**, 1303–1316. (doi:10.1084/jem.20011624)
40. Huber V *et al.* 2005 Human colorectal cancer cells induce T-Cell death through release of proapoptotic microvesicles: role in immune escape. *Gastroenterology* **128**, 1796–1804. (doi:10.1053/j.gastro.2005.03.045)
41. Liu Z-M, Wang Y-B, Yuan X-H. 2013 Exosomes from murine-derived GL26 cells promote glioblastoma tumor growth by reducing number and function of CD8+T cells. *Asian Pac. J. Cancer Prev.* **14**, 309–314. (doi:10.7314/APJCP.2013.14.1.309)
42. Rong L, Li R, Li S, Luo R. 2016 Immunosuppression of breast cancer cells mediated by transforming growth factor-beta in exosomes from cancer cells. *Oncol. Lett.* **11**, 500–504.
43. Peinado H *et al.* 2012 Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* **18**, 883–891. (doi:10.1038/nm.2753)
44. Gross JC, Chaudhary V, Bartscherer K, Boutros M. 2012 Active Wnt proteins are secreted on exosomes. *Nat. Cell Biol.* **14**, 1036–1045. (doi:10.1038/ncb2574)
45. Boyiadzis M, Whiteside TL. 2015 Information transfer by exosomes: a new frontier in hematologic malignancies. *Blood Rev.* **29**, 281–290. (doi:10.1016/j.blre.2015.01.004)
46. Xiang X *et al.* 2009 Induction of myeloid-derived suppressor cells by tumor exosomes. *Int. J. Cancer* **124**, 2621–2633. (doi:10.1002/ijc.24249)
47. Bubenik J. 2004 MHC class I down-regulation: tumour escape from immune surveillance? (review). *Int. J. Oncol.* **25**, 487–491.

48. Ljunggren HG, Karre K. 1990 In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol. Today* **11**, 237–244. (doi:10.1016/0167-5699(90)90097-S)
49. Clayton A, Mitchell JP, Court J, Linnane S, Mason MD, Tabi Z. 2008 Human tumor-derived exosomes down-modulate NKG2D expression. *J. Immunol.* **180**, 7249–7258. (doi:10.4049/jimmunol.180.11.7249)
50. Groh V, Wu J, Yee C, Spies T. 2002 Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* **419**, 734–738. (doi:10.1038/nature01112)
51. Hedlund M, Nagaeva O, Kargl D, Baranov V, Mincheva-Nilsson L. 2011 Thermal- and oxidative stress causes enhanced release of NKG2D ligand-bearing immunosuppressive exosomes in leukemia/lymphoma T and B cells. *PLoS ONE* **6**, e16899. (doi:10.1371/journal.pone.0016899)
52. Hong CS, Muller L, Boyiadzis M, Whiteside TL. 2014 Isolation and characterization of CD34+ blast-derived exosomes in acute myeloid leukemia. *PLoS ONE* **9**, e103310. (doi:10.1371/journal.pone.0103310)
53. Lundholm M, Schroder M, Nagaeva O, Baranov V, Widmark A, Mincheva-Nilsson L. 2014 Prostate tumor-derived exosomes down-regulate NKG2D expression on natural killer cells and CD8+ T cells: mechanism of immune evasion. *PLoS ONE* **9**, e108925. (doi:10.1371/journal.pone.0108925)
54. Gilkes DM, Semenza GL, Wirtz D. 2014 Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nat. Rev. Cancer* **14**, 430–439. (doi:10.1038/nrc3726)
55. Berchem G *et al.* 2016 Hypoxic tumor-derived microvesicles negatively regulate NK cell function by a mechanism involving TGF-beta and miR23a transfer. *Oncoimmunology* **5**, e1062968. (doi:10.1080/2162402X.2015.1062968)
56. Su M-W, Yu S-L, Lin W-C, Tsai C-H, Chen P-H, Lee YL. 2016 Smoking-related microRNAs and mRNAs in human peripheral blood mononuclear cells. *Toxicol. Appl. Pharmacol.* **305**, 169–175. (doi:10.1016/j.taap.2016.06.020)
57. Skog J *et al.* 2008 Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat. Cell Biol.* **10**, 1470–1476. (doi:10.1038/ncb1800)
58. Ding G *et al.* 2015 Pancreatic cancer-derived exosomes transfer miRNAs to dendritic cells and inhibit RFXAP expression via miR-212-3p. *Oncotarget* **6**, 29 877–29 888. (doi:10.18632/oncotarget.4924)
59. Ye S-B, Li Z-L, Luo D-H, Huang B-J, Chen Y-S, Zhang X-S, Cui J, Zeng Y-X, Li J. 2014 Tumor-derived exosomes promote tumor progression and T-cell dysfunction through the regulation of enriched exosomal microRNAs in human nasopharyngeal carcinoma. *Oncotarget* **5**, 5439–5452. (doi:10.18632/oncotarget.2118)
60. Reiners KS, Shatnyeva O, Vasyutina E, Bösl T, Hansen HP, Hallek M, Herling M, Strandmann EP. 2017 Extracellular vesicles released from chronic lymphocytic leukemia cells exhibit a disease relevant mRNA signature and transfer mRNAs to bystander cells. *Haematologica* **102**, e100–e103. (doi:10.3324/haematol.2016.153197)
61. Muller L, Mitsuhashi M, Simms P, Gooding WE, Whiteside TL. 2016 Tumor-derived exosomes regulate expression of immune function-related genes in human T cell subsets. *Sci. Rep.* **6**, 20254. (doi:10.1038/srep20254)
62. Antonioli L, Fornai M, Colucci R, Ghisu N, Tuccori M, Del Tacca M, Blandizzi C. 2008 Pharmacological modulation of adenosine system: novel options for treatment of inflammatory bowel diseases. *Inflamm. Bowel Dis.* **14**, 566–574. (doi:10.1002/ibd.20316)
63. Kumar V, Sharma A. 2009 Adenosine: an endogenous modulator of innate immune system with therapeutic potential. *Eur. J. Pharmacol.* **616**, 7–15. (doi:10.1016/j.ejphar.2009.05.005)
64. Clayton A, Al-Taei S, Webber J, Mason MD, Tabi Z. 2011 Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production. *J. Immunol.* **187**, 676–683. (doi:10.4049/jimmunol.1003884)
65. Soderberg A, Barral AM, Soderstrom M, Sander B, Rosen A. 2007 Redox-signaling transmitted *in trans* to neighboring cells by melanoma-derived TNF-containing exosomes. *Free Radic. Biol. Med.* **43**, 90–99. (doi:10.1016/j.freeradbiomed.2007.03.026)
66. Viaud S, Thery C, Ploix S, Tursz T, Lapierre V, Lantz O, Zitvogel L, Chaput N. 2010 Dendritic cell-derived exosomes for cancer immunotherapy: what's next? *Cancer Res.* **70**, 1281–1285. (doi:10.1158/0008-5472.CAN-09-3276)
67. Lamparski HG, Metha-Damani A, Yao J-Y, Patel S, Hsu D-H, Ruegg C. 2002 Production and characterization of clinical grade exosomes derived from dendritic cells. *J. Immunol. Methods* **270**, 211–226. (doi:10.1016/S0022-1759(02)00330-7)
68. Robbins PD, Morelli AE. 2014 Regulation of immune responses by extracellular vesicles. *Nat. Rev. Immunol.* **14**, 195–208. (doi:10.1038/nri3622)
69. Pitt JM, Charrier M, Viaud S, Andre F, Besse B, Chaput N, Zitvogel L. 2014 Dendritic cell-derived exosomes as immunotherapies in the fight against cancer. *J. Immunol.* **193**, 1006–1011. (doi:10.4049/jimmunol.1400703)
70. Krishnadas DK, Bai F, Lucas KG. 2013 Cancer testis antigen and immunotherapy. *Immunotargets Ther.* **2**, 11–19. (doi:10.2147/ITT.S35570)
71. Escudier B *et al.* 2005 Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial. *J. Transl. Med.* **3**, 10. (doi:10.1186/1479-5876-3-10)
72. Morse MA *et al.* 2005 A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. *J. Transl. Med.* **3**, 9. (doi:10.1186/1479-5876-3-9)
73. Segura E *et al.* 2005 ICAM-1 on exosomes from mature dendritic cells is critical for efficient naive T-cell priming. *Blood* **106**, 216–223. (doi:10.1182/blood-2005-01-0220)
74. Utsugi-Kobukai S, Fujimaki H, Hotta C, Nakazawa M, Minami M. 2003 MHC class I-mediated exogenous antigen presentation by exosomes secreted from immature and mature bone marrow derived dendritic cells. *Immunol. Lett.* **89**, 125–131. (doi:10.1016/S0165-2478(03)00128-7)
75. Viaud S *et al.* 2011 Updated technology to produce highly immunogenic dendritic cell-derived exosomes of clinical grade: a critical role of interferon-gamma. *J. Immunother.* **34**, 65–75. (doi:10.1097/CJI.0b013e3181fe535b)
76. Segura E, Amigorena S, Thery C. 2005 Mature dendritic cells secrete exosomes with strong ability to induce antigen-specific effector immune responses. *Blood Cells Mol. Dis.* **35**, 89–93. (doi:10.1016/j.bcmd.2005.05.003)
77. Besse B *et al.* 2016 Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. *Oncoimmunology* **5**, e1071008. (doi:10.1080/2162402X.2015.1071008)
78. Pogge von Strandmann E *et al.* 2007 Human leukocyte antigen-B-associated transcript 3 is released from tumor cells and engages the NKp30 receptor on natural killer cells. *Immunity* **27**, 965–974. (doi:10.1016/j.immuni.2007.10.010)
79. Binici J, Koch J. 2014 BAG-6, a jack of all trades in health and disease. *Cell. Mol. Life Sci.* **71**, 1829–1837. (doi:10.1007/s00018-013-1522-y)
80. Kamper N, Franken S, Temme S, Koch S, Bieber T, Koch N. 2012 Gamma-Interferon-regulated chaperone governs human lymphocyte antigen class II expression. *FASEB J.* **26**, 104–116. (doi:10.1096/fj.11-189670)
81. Corduan A, Lecomte S, Martin C, Michel D, Desmots F. 2009 Sequential interplay between BAG6 and HSP70 upon heat shock. *Cell. Mol. Life Sci.* **66**, 1998–2004. (doi:10.1007/s00018-009-9198-z)
82. Thress K, Song J, Morimoto RI, Kornbluth S. 2001 Reversible inhibition of Hsp70 chaperone function by Scythe and Reaper. *EMBO J.* **20**, 1033–1041. (doi:10.1093/emboj/20.5.1033)
83. Massa C, Melani C, Colombo MP. 2005 Chaperon and adjuvant activity of hsp70: different natural killer requirement for cross-priming of chaperoned and bystander antigens. *Cancer Res.* **65**, 7942–7949. (doi:10.1158/0008-5472.CAN-05-0377)
84. Gastpar R, Gehrmann M, Bausero MA, Asea A, Gross C, Schroeder JA, Multhoff G. 2005 Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. *Cancer Res.* **65**, 5238–5247. (doi:10.1158/0008-5472.CAN-04-3804)
85. Reiners KS *et al.* 2013 Soluble ligands for NK cell receptors promote evasion of chronic lymphocytic leukemia cells from NK cell anti-tumor activity. *Blood* **121**, 3658–3665. (doi:10.1182/blood-2013-01-476606)
86. Simhadri VR *et al.* 2008 Dendritic cells release HLA-B-associated transcript-3 positive exosomes to regulate natural killer function. *PLoS ONE* **3**, e3377. (doi:10.1371/journal.pone.0003377)
87. Gross C, Hansch D, Gastpar R, Multhoff G. 2003 Interaction of heat shock protein 70 peptide with NK cells involves the NK receptor CD94. *Biol. Chem.* **384**, 3757–3779. (doi:10.1515/BC.2003.030)

88. Gross C, Schmidt-Wolf IG, Nagaraj S, Gastpar R, Ellwart J, Kunz-Schughart LA, Multhoff G. 2003 Heat shock protein 70-reactivity is associated with increased cell surface density of CD94/CD56 on primary natural killer cells. *Cell Stress Chaper*. **8**, 348.
89. Barchet W, Wimmenauer V, Schlee M, Hartmann G. 2008 Accessing the therapeutic potential of immunostimulatory nucleic acids. *Curr. Opin. Immunol.* **20**, 389–395. (doi:10.1016/j.coi.2008.07.007)
90. Hornung V *et al.* 2006 5′-Triphosphate RNA is the ligand for RIG-I. *Science* **314**, 994–997. (doi:10.1126/science.1132505)
91. Schlee M *et al.* 2009 Recognition of 5′ triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. *Immunity* **31**, 25–34. (doi:10.1016/j.immuni.2009.05.008)
92. Dassler-Plenker J *et al.* 2016 RIG-I activation induces the release of extracellular vesicles with antitumor activity. *Oncoimmunology* **5**, e1219827. (doi:10.1080/2162402X.2016.1219827)
93. Brandt CS *et al.* 2009 The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans. *J. Exp. Med.* **206**, 1495–1503. (doi:10.1084/jem.20090681)
94. Kaifu T, Escaliere B, Gastinel LN, Vivier E, Baratin M. 2011 B7-H6/NKp30 interaction: a mechanism of alerting NK cells against tumors. *Cell. Mol. Life Sci.* **68**, 3531–3539. (doi:10.1007/s00018-011-0802-7)
95. Baratin M, Vivier E. 2010 B7-H6: Un nouveau signal d'alarme pour les cellules natural killer. *Med. Sci.* **26**, 119–120.
96. Banchereau J, Steinman RM. 1998 Dendritic cells and the control of immunity. *Nature* **392**, 245–252. (doi:10.1038/32588)
97. Bubnoff D, Scheler M, Wilms H, Fimmers R, Bieber T. 2011 Identification of IDO-positive and IDO-negative human dendritic cells after activation by various proinflammatory stimuli. *J. Immunol.* **186**, 6701–6709. (doi:10.4049/jimmunol.1003151)
98. Matta J *et al.* 2013 Induction of B7-H6, a ligand for the natural killer cell-activating receptor NKp30, in inflammatory conditions. *Blood* **122**, 394–404. (doi:10.1182/blood-2013-01-481705)