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# Exosomal lncRNAs and cancer: connecting the missing links

Hojjat Naderi-Meshkin[1,3], Xin Lai[2], Raheleh Amirkhah[3,4], Julio Vera[2], John EJ Rasko[5,6,7], Ulf Schmitz[5,6*]

[1]*Stem Cells and Regenerative Medicine Research Group, Academic Center for Education, Culture Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran,* [2]*Laboratory of Systems Tumour Immunology, Department of Dermatology, Friedrich-Alexander-University of Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Germany,* [3]*Nastaran Center for Cancer Prevention, Mashhad, Iran,* [4]*Reza Institute of Cancer Bioinformatics and Personalized Medicine, Mashhad, Iran,* [5]*Gene and Stem Cell Therapy Program, Centenary Institute, Camperdown, Australia,* [6]*Sydney Medical School, University of Sydney, Sydney, Australia,* [7]*Cell and Molecular Therapies, Royal Prince Alfred Hospital, Camperdown, Australia*

*To whom correspondence should be addressed.

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## Abstract

**Motivation:** Extracellular vesicles (EVs), including exosomes and microvesicles, are potent and clinically valuable tools for early diagnosis, prognosis and potentially the targeted treatment of cancer. The content of EVs is closely related to the type and status of the EV-secreting cell. Circulating exosomes are a source of stable RNAs including mRNAs, microRNAs, and long noncoding RNAs (lncRNAs).

**Results:** This review outlines the links between EVs, lncRNAs, and cancer. We highlight communication networks involving the tumor microenvironment, the immune system, and metastasis. We show examples supporting the value of exosomal lncRNAs as cancer biomarkers and therapeutic targets. We demonstrate how a system biology approach can be used to model cell-cell communication via exosomal lncRNAs and to simulate effects of therapeutic interventions. In addition, we introduce algorithms and bioinformatics resources for the discovery of tumor-specific lncRNAs and tools that are applied to determine exosome content and lncRNA function. Finally, this review provides a comprehensive collection and guide to databases for exosomal lncRNAs.

**Contact:** u.schmitz@centenary.org.au

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## 1 Introduction

In recent years, EVs have drawn great interest, because of their potential as disease biomarkers, therapeutic targets, and vehicles for drug delivery. There are many types of EVs, which are different in size, morphology, biogenesis, and mechanisms by which they are released (Maas, et al., 2017). Cell-derived EVs can be broadly classified into: (1) small nano-sized exosomes that are formed within the cytoplasm by inward budding of endosomes and then pooled into multivesicular bodies (MVBs). MVBs in turn fuse with the cell membrane and release the exosomes into the extracellular space; (2) nano to micro-sized vesicles (or microvesicles), which are shed from the plasma membrane; and (3) micro-sized vesicles created as byproducts of cell death (apoptotic bodies). The classes of EVs are summarized in Supplementary Table 1. Virtually all cells produce EVs. They carry different cargo depending on the cell type of origin, and their number and content change in response to pathophysiological states and micro-environmental stimuli (Quesenberry, et al., 2015). EVs deliver a variety of biomolecules including lipids, proteins, DNA, mRNA, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) to recipient cells. Once recipient cells are reached, the possible fates of vesicular content include its degradation, release into the recipient cell's cytoplasm, or even transport into the nucleus. Vesicle exchange among cells can be dynamic and bidirectional, so as to allow response to, and coordination of, biological events (Quesenberry, et al., 2014). Methods to isolate and analyze EVs have been discussed previously in (Mateescu, et al., 2017).

LncRNAs are a heterogeneous group of noncoding transcripts localized in different cellular compartments. LncRNAs are longer than 200 nucleotides and can be coarsely classified into intragenic (intronic or antisense) and intergenic lncRNAs (Schmitz, et al., 2015). Some lncRNAs are stable, vastly expressed, and highly conserved, while others have a high turnover, are barely detectable, and poorly conserved. LncRNAs have diverse biological functions, e.g. coordinating gene regulation at DNA and RNA level, or interfering with protein biogenesis and function (Schmitz, et al., 2015). They have been shown to be involved in chromatin remodeling, transcriptional control, and post-transcriptional processing. To exert their function, lncRNAs interact with DNA, RNA, and proteins in both sequence-specific and conformational engagements acting as scaffolds, decoys (molecular sink/miRNA sponge), and enhancer RNAs. Many lncRNAs can be found in human body fluids within circulating tumor cells or EVs. These protected lncRNAs are rather stable; providing the opportunity to easily extract them from blood or other body fluids and use them as biomarkers for disease diagnosis, prognosis, and therapy. (Li, et al., 2015). Recent studies suggest that exosomes and lncRNAs might function together to disseminate molecules and signals for the purpose of changing or regulating recipient cells of various types locally or at distance.

In this review, which is intended for both biologists and bioinformaticians, we provide an overview about the importance of exosomes and lncRNAs in cancer as well as current computational tools and databases to characterize the roles of exosomes and lncRNAs in human malignancies.

## 2 The importance of exosomes and lncRNAs in cancer

An exosome secreted from a malignant cell often reflects the molecular composition, and therefore reveals the pathophysiological status, of its cell of origin (Lobb, et al., 2017). Exosomes loading is a selective process. Cancer cells and tumor-reactive immune cells, for example, release exosomes into the bloodstream or other body fluids to deliver specific paracrine or endocrine messages (Figure 1). In cancer, exosome content is produced to regulate diverse pivotal processes and functions of tumor cells such as growth, proliferation, survival, migration, neoangiogenesis, immunomodulatory functions and anti-cancer drug resistance (Ma, et al., 2017). Tumor cells can thereby modify the immune context of their microenvironment to escape from immunosurveillance(Ye, et al., 2014), induce stromal remodeling, and mediate pre-metastatic niche formation in selected host tissues (Vallabhaneni, et al., 2017). Several studies have shown that exosomes play key roles in establishing pre-metastatic niches by horizontal transfer of malignant traits (Costa-Silva, et al., 2015). Exosomes exhibit a distinct pattern of integrins on their surface that lets them fuse with recipient cells at specific destinations. For example, exosomal αvβ5 integrin was linked to liver metastasis, while α6β4 and α6β1 integrins were associated with lung metastasis (Hoshino, et al., 2015). Yu et al. found that exosomes from highly metastatic pancreatic cancer cells contained differentially expressed proteins that are involved in exosome-mediated intercellular communication (Yu, et al., 2017). Abdouh *et al.* have shown that recipient cells harboring a tumor suppressor mutation show increased uptake of primary tumor-derived exosomes (Abdouh, et al., 2017). This is mediated by the overexpression and/or *de novo* expression of proteins and surface receptors responsible for the interaction with oncogenic factor-containing exosomes. Tumor cells, immune cells, and stromal cells reciprocally exchange exosomes. Tumor-derived exosomes are able to educate dendritic cells to drive metastasis (Shen, et al., 2017) and can also promote metastasis by educating both bone marrow and pre-metastatic niche in favor of primary tumor cell hosting (Peinado, et al., 2012). RNA interference of RAB27A, which is responsible for exosome release and membrane trafficking, reduced exosome production, and decreased tumor growth and metastasis in melanoma cells (Peinado, et al., 2012). In other scenarios, exosomes are released from tumor cells to increase resistance to chemotherapy. Boelens et al. demonstrated that stromal and breast cancer cells utilize exosomes to induce resistance to chemotherapy and radiation (Boelens, et al., 2014). Pretreatment with an inhibitor of exosome release (Ketotifen), on the other hand, can sensitize tumor cells to the anticancer drug Doxorubicin (Khan, et al., 2018).

Tumor-derived exosomes carry molecular signatures comprising effectors of different stages of tumor progression. Such signatures can provide valuable biomarkers with potential diagnostic or prognostic value (Barile and Vassalli, 2017). Additionally, exosomes can be used to predict or monitor patients' response to treatment (Takahashi, et al., 2014). Monitoring of cancers by biopsy does not allow for frequent sampling. In contrast, monitoring of exosomes in body fluids facilitates frequent non- or minimally invasive sampling via liquid biopsy. Based on the type and location of the tumor, exosomes could be isolated from plasma/serum, urine, cerebrospinal fluid, saliva etc. (Iwai, et al., 2016). Castillo et al. revealed that surfaceome profiling of exosomes enables cancer-specific molecular profiling of exosomal cargo by noninvasive liquid biopsy (Castillo, et al., 2018).

The capacity of circulating exosomes to stably maintain and deliver biomolecules makes these nano- and micro-particles attractive carriers of therapeutic agents (Jiang, et al., 2017). Exosomes could be used as a vehicle to selectively deliver therapeutic drugs for cancer therapy similar

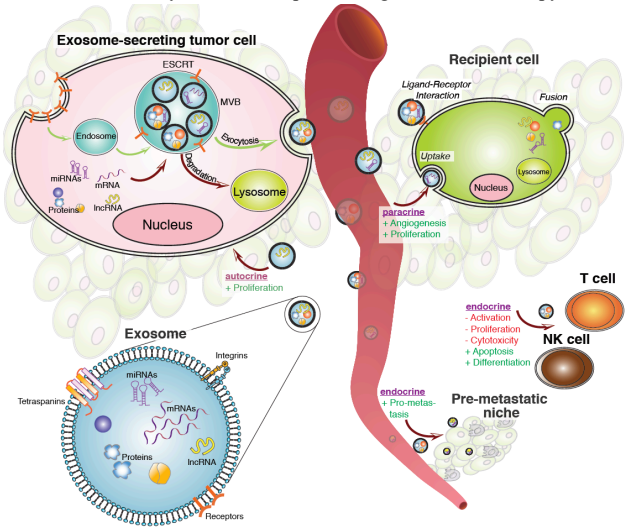


**Figure 1 Schematic of cargo transfer by exosomes.** A specific molecular machinery called the endosomal sorting complex required for transport (ESCRT) is involved in sorting and incorporation of material into multivesicular bodies (MVBs). Components of secretory cells are selectively incorporated into MVBs (top left). MVBs may either fuse with lysosomes for component degradation or fuse with the plasma membrane for exocytosis (release of exosomes into the extracellular milieu). Exosomes contain diverse cargoes, such as membrane-bound and cytosolic proteins, transcription factors, mRNAs, lncRNAs, and miRNAs, etc (see magnified exosome - bottom left). At recipient cells, exosomes may either fuse directly with the plasma membrane or are endocytosed (top right). Both mechanisms result in the delivery of proteins and RNAs into the membrane or cytosol of recipient cells. Exosomes may also interact with target cells by direct signaling through ligands/receptors on their respective surfaces. Within the target cell, exosomal lncRNAs might act as RNA decoys of transcription, scaffolds to form ribonucleoprotein complexes (RNP), miRNA sponges, chromatin modifiers, splicing modulators, translation inhibitors, or mRNA degradation signals. Exosomes can travel through body fluids to distal sites. In cancer, this system of delivering endocrine messages is used to establish pre-metastatic niches (bottom right). Whether sent as autocrine, paracrine, or endocrine messages, exosomes can alter the characteristics of their recipient cells, e.g. to suppress unwanted functions (highlighted in red) or induce favorable processes (green).

to minicells (MacDiarmid, et al., 2007), a platform for the targeted delivery of chemotherapeutics and functional nucleic acids in cancer patented by EnGeneIC Ldt. Several studies have provided evidence that exosomes can deliver exogenous RNA, including small interfering RNAs (siRNAs) and miRNAs, to target cells and can be functional both *in vitro* and *in vivo*. A comprehensive overview of these studies was published recently in (Johnsen, et al., 2014). Smyth et al. showed that exosomes represent specificity toward tumor cells 10 fold more than liposomes and cleavage of exosome surface proteins give rise to diminished association with their target cell (Smyth, et al., 2014).

Compared to synthetic drug delivery systems, exosomes have natural advantages such as their innate stability, being non-toxic, or being immune privileged. This is particularly important when it comes to the design of personalized therapeutic interventions. Toda et al., for example, demonstrated that cell tropism is important for glioblastoma exosome uptake by parental cells as well as other cancer cell lines (Toda, et al., 2015). Alvarez-Erviti et al. were the first to show that exosomes can cross biological barriers, such as the blood-brain barrier (Alvarez-Erviti, et al., 2011). Aqil and co-authors have shown that exosomal formulation of drugs enhances their anti-tumor efficacy in lung cancer and reduces off-site toxicity (Aqil, et al., 2016). Kamerkar et al. engineered normal fibroblast-like mesenchymal cell-derived exosomes to carry KRAS-specific short interfering RNA and showed that these so called iExosomes efficiently target KRAS in pancreatic cancer and thereby increase overall survival (Kamerkar, et al., 2017). In addition, enhanced retention of iExosomes was observed in the presence of CD47 suppressing exosomal phagocytosis. Interestingly, even behavioral factors such as social support of cancer patients could change the transcriptome of exosomes, suggesting the possibility of leveraging exosomes to improve personalized medicine (Lutgendorf, et al., 2018).

LncRNAs have roles in both oncogenic and tumor suppressive pathways. For example, lncRNA SPRY4-IT1 plays an oncogenic role in bladder cancer by acting as miR-101-3p sponge and thereby positively regulating the expression of EZH2, which in turn promotes proliferation and metastasis (Liu, et al., 2017). ANCR, in contrast, is an lncRNA that acts as tumor suppressor mediating the degradation of EZH2 to attenuate the invasion and metastasis of breast cancer (Li, et al., 2017). LncRNAs are expressed abundantly in cancer cells and show greater tissue-specificity compared to protein-coding mRNAs. Thus, expression levels of lncRNAs directly correlate with cancer phenotypes and therefore represent a better biomarker. Similar to miRNAs, they exhibit distinct expression patterns in primary tumors and metastases and play pivotal roles in mediating pathogenic mechanisms of cancers. Their abundance is specifically regulated in response to signals such as DNA damage (Sharma, et al., 2015), chemokines (Xing, et al., 2014), and environmental stresses (Zeng, et al., 2016).

LncRNA expression can be blocked by siRNAs, antisense oligonucleotides (ASO), hammerhead ribozymes, aptamers, and small molecules (Li and Chen, 2013). Targeting lncRNAs for cancer therapy can also take advantage of the fact that some lncRNAs function by forming a scaffold to bind or recruit protein complexes to specific genomic loci (Tsai, et al., 2010). Small molecule drugs, for example, by structure-specific docking to the target lncRNAs, can be selectively used for blocking the function of oncogenic lncRNAs (Li and Chen, 2013). Xia et al. suggested lncRNA ASBEL as a novel therapeutic target in triple-negative breast cancer. They showed that an anti-ASBEL ASO can effectively downregulate ASBEL, which in turn leads to increased BTG3 expression - an anti-proliferation protein (Xia, et al., 2017). Similarly, Arun et al. showed that knockdown of Malat1 lncRNA expression in a mouse model of breast cancer by a specific ASO suppresses metastasis (Arun, et al., 2016). Recently, Ozes et al. have developed an anti-lncRNA peptide nucleic acid that effectively blocks the HOTAIR-EZH2 interaction to inhibit ovarian and breast cancer cell invasion, and increase chemotherapy sensitivity (Ozes, et al., 2017).

Modulating lncRNA expression is one approach to decipher their roles in cancers. Although, ASOs are the current gold standard used to determine lncRNA function and to develop lncRNAs-based cancer therapies, new strategies based on CRISPRi to interfere and CRISPRa to activate lncRNA transcription are novel potent approaches to study lncRNA functions (Liu, et al., 2017; Shechner, et al., 2015).

## 3 Exosomal lncRNA-based biomarkers and therapeutic targets

Exosomal lncRNA profiling can be used for the identification of cancer subtypes, biomarkers as well as potential therapeutic targets. The most prominent example of an lncRNA biomarker is PCA3, which is derived from patient's urine and is more specific in diagnosing prostate cancer than the commonly used prostate-specific antigen (PSA). PCA3 already found wide clinical application (Lee, et al., 2011). Ren et al. have reported that a lncRNA fragment called MALAT1-derived miniRNA (MD-miniRNA) can be used as plasma-based biomarker for prostate cancer diagnosis with improved diagnostic accuracy compared to PSA (Ren, et al., 2013). Zhang et al. found lncRNAs HOTAIR, MALAT1, and MEG3 differentially expressed in cervical cancer-derived exosomes and highlighted their potential as biomarkers (Zhang, et al., 2016). Recently, a serum-circulating lncRNA signature was found to discriminate patients with clear cell renal cell carcinoma from healthy individuals (Wu, et al., 2016). Pan et al. indicated that exosomal lncRNA ZFAS1 mediates gastric cancer progression and proposed ZFAS1 as a diagnostic and prognostic biomarker for gastric cancer (Pan, et al., 2017). Zhang et al. found that the levels of exosomal MALAT1 in serum of non-small cell lung cancer patients were positively related to tumor stage and lymphatic metastasis, suggesting exosomal MALAT1 as a non-invasive serum-based biomarker for diagnosis and prognosis for non-small cell lung cancer (Zhang, et al., 2017). Interestingly, another group found that exosomal MALAT1 is also associated with disease progression in breast cancer (Zhang, et al., 2018). Gao et al. found that the colorectal cancer patients with high expression of exosomal lncRNA 91H showed a higher risk in tumor development; suggesting it as an early plasma-based diagnostic biomarker for colorectal cancer recurrence and metastasis (Gao, et al., 2018). Duan et al. have introduced a panel of lncRNAs (MEG3, SNHG16 and MALAT1) for the diagnosis and recurrence prediction of bladder cancer, with significantly higher diagnostic performance than urine cytology (Duan, et al., 2016). Using RNA-sequencing, Berrondo et al. have identified three known lncRNAs (HOTAIR, HYMA1, and OTX2-AS1), and two novel lncRNAs (LINC00477 and LOC100506688) that were enriched in urinary exosomes of urothelial bladder cancer (Berrondo, et al., 2016). Dong et al. quantified lncRNAs in three types of serum EVs (apoptotic bodies, microvesicles, and exosomes) and found that exosomes contain the richest reservoir of almost all measured circulating lncRNAs. The authors proposed a combination of two mRNAs and one lncRNA (BCAR4) as biomarkers for the detection of colorectal cancer (Dong, et al., 2016). Bioinformatics analyses have found an overrepresentation of exosomal lncRNAs with miRNA response elements in prostate cancer and hepatocellular carcinoma (Ahadi, et al., 2016; Li, et al., 2018). In this context, FAL1 has been shown to sponge

miR-1236 and thereby promote cell proliferation and migration in hepatocellular carcinomas (Li, et al., 2018).

Exosomal RNA-based biomarkers are *en route* to clinical application. This is evidenced by several current clinical trials. Researchers at the Ruijin Hospital in Shanghai (China), for example, are identifying biomarkers for lung metastases of primary high-grade osteosarcoma based on RNA profiles in circulating exosomes (NCT03108677). Similarly, in another recently launched clinical trial (NCT03102268) at The Second Affiliated Hospital of the Nanjing Medical University (China) the goal is to examine the ncRNAs of cholangiocarcinoma derived exosomes for their diagnostic and predictive value.

The mechanism for selectively loading lncRNAs into exosomes is currently unknown. Hewson et al. found that certain lncRNAs are preferentially packaged into exosomes, and that the abundance of exosomal RNA transcripts, including lncRNAs, correlates with their expression in the cell of origin (Hewson, et al., 2016). Interestingly, Kogure et al. found that the expression of several ultraconserved lncRNAs, especially TUC339, were dramatically increased in exosomes compared to their respective hepatocellular cancer cell lines (Hep3B and PLC/PRF/5 HCC cells); supporting the existence of selective packaging mechanisms for lncRNA export. siRNA-mediated knockdown of TUC339 in tumor cells decreased cell proliferation and clonogenic growth of hepatocellular cancer indicating that tumor cells can exert their genetic influences by exosome-mediated intercellular transfer of lncRNA (Kogure, et al., 2013). In another study, Koldemir et al. have demonstrated that even a small increase in cellular expression of lncRNA GAS5 leads to a significant enrichment of GAS5 in exosomes (Koldemir, et al., 2017).

Exosome-mediated shuttling of lncRNAs in pathological conditions, such as cancer, differs from normal physiology both in levels and types of lncRNAs that are transported. LncRNAs encapsulated in tumor-derived exosomes can modulate the function of recipient cells, promoting growth, cell survival, and chemoresistance in recipient cells or modulate immune responses (Figure 1). For example, Takahashi et al. have shown in two different studies that exosome-mediated transfer of linc-RoR and linc-VLDLR can modulate the sensitivity of tumor cells to chemotherapy in human hepatocellular cancer (Takahashi, et al., 2014; Takahashi, et al., 2014). Tumor cells through transferring exosomal linc-RoR can promote cancer progression by inducing cell survival under hypoxic stress in hepatocellular cancer (Takahashi, et al., 2014). Xue et al. showed that hypoxic exosomes secreted from primary tumors into the tumor microenvironment facilitate tumor growth and development in bladder cancer through the transfer of oncogenic lncRNA UCA1 (Xue, et al., 2017). In another study, exosome-mediated transfer of UCA1 resulted in enhanced Tamoxifen resistance in breast cancer cells (Xu, et al., 2016). Similarly, Qu et al. indicated that exosome-transmitted lncARSR contributes to tumor progression by promoting Sunitinib resistance, which is a major challenge in the treatment of advanced renal cancer (Qu, et al., 2016).

Tumor cells can also modulate cells in their surrounding microenvironment through exosomal lncRNAs and thereby facilitate spreading into secondary tumor sites and escaping from immune control (Figure 1). Lang et al. showed that glioma cells can promote angiogenesis through the release of exosomes enriched in lncRNA POU3F3 (Lang, et al., 2017). Similar effects are achieved by exosomes enriched in lncRNA CCAT2 (Lang, et al., 2017). Huan et al. revealed that exosomes play a role in developing the niche in the bone marrow of acute myelogenous leukemia patients by transferring both coding and noncoding RNAs into bystander cells (Huan, et al., 2013). Another study indicated that exosomes derived from epithelial ovarian cancer can remotely restore the migration potential of endothelial cells by transferring lncRNAs, which was previously suppressed by tumor associated macrophage-derived exosomes (Wu, et al., 2017).

The therapeutic use of exosome-mediated lncRNAs remains relatively unexplored despite possible advantages. Some lncRNAs have been characterized to act as tumor suppressors, which could be used in adjuvant therapeutic settings. For example, GAS5 (growth arrest-specific 5) is a tumor suppressor lncRNA, which reduces cell growth and metabolism, and mediates apoptosis (Kino, et al., 2010). As a therapeutic approach, tumor-suppressor lncRNAs could be selectively increased through an exosome-based homing system. While not yet reported for exosome-based targeted delivery of lncRNAs, EV-secreting cells can be genetically modified to express targeting ligands to specifically deliver therapeutics to tumor cells (Kooijmans, et al., 2016).

However, many challenges regarding lncRNAs in exosomes need to be confronted. For example, using bioinformatics and experimental validation, functions of lncRNAs and their specificity for a particular cancer must be determined. Another major challenge is the lack of tools to specifically induce or interfere with EV release, without affecting the release of other EV subtypes (Gezer, et al., 2014). In the clinical context, the preparation of exosomal lncRNAs from biofluids and qRT-PCR-based quantification need to be standardized to be able to generalize findings from different patients, groups, and labs. Prior to the application in a clinical context the establishment of international reference standards and laboratory accreditation programs will be needed. A good example of how this standardization process could be conducted is provided by the work of Hughes and co-authors. In their essay, they describe the qRT-PCR-based lab diagnosis and monitoring of BCR-ABL1 in chronic myeloid leukemia patients (Hughes, et al., 2006).

## 4 Mathematical modeling of cancer cell communication

Mathematical modeling approaches have previously been adopted to understand mechanisms underlying eukaryotic cell-cell communication. Jolly et al., for example, used a system of ordinary differential equations to model the role that Notch–Delta–Jagged signaling plays in regulating cell-cell communication systems and in determining cell fates (Jolly, et al., 2015). The authors suggest that a hybrid cell fate can be induced via Notch–Delta–Jagged signaling, in which cells can act both as sender and receiver in a cell-cell communication system. This hybrid sender/receiver fate allows cancer cells to move and circulate collectively promoting cancer metastasis by enabling collective cell migration and expanding the cancer stem cell population. More recently, a mechanistic model composed of ordinary differential equations was developed to study cancer cell population dynamics in the interplay between leukemic blast cells and immune cells in acute myeloid leukemia (Nishiyama, et al., 2018). The model, parameterized with clinical data, has two stable steady states, corresponding to (i) high leukemic cell load at diagnosis/relapse, and (ii) low leukemic cell load at remission. Transitions between these steady states are supposedly induced by chemotherapy and immunotherapy. Santos *et al.* combined kinetic modeling and patient gene expression data analysis to elucidate biological mechanisms by which melanoma becomes resistant to the immune attack and to immunotherapy (Santos, et al., 2016). Their model simulations suggest that an immunotherapy can be improved through the co-administration of cytokines. Wenbo and Wang proposed a network model of cancer tumorigenesis involving the immune microenvironment (Wenbo and Wang, 2017). The model includes cancer cells, 12 types of immune cells and 13 types of cytokines, and describes processes involving cell–cell interactions, cytokine–cell interactions and cell–cytokine production. Their

model simulations suggest that tumorigenesis and cancer recovery processes may need to go through cancer–immune oscillation involving escape, elimination and equilibrium phases in immunoediting. Li developed a similar model of a cancer-immunity interaction network to study the landscape of stochastic dynamics and kinetic transitions of the cancer–immunity interaction system (Li, 2017). Li discovered key interaction links using sensitivity analysis and predicted anticancer therapeutic targets based on optimizing cancer-to-immune state transition actions. Gong *et al.* proposed a multi-scale agent-based modeling approach as beneficial in studying interactions between immune cells and cancer cells since it is able to include the distribution of cell populations in the 3D space and examine intra-tumoral heterogeneity. Their simulations reproduce spatial patterns of PDL1 expression resembling immunoarchitecture found in patient biopsies. Kim and Lee used a hybrid agent-based and delay differential equation approach to model protective antitumor immunity via cancer vaccines (Kim and Lee, 2012). Model parameters include cell proliferation rates, tumor antigenicity, recruitment times of cytotoxic T lymphocytes, and initial memory cytotoxic T lymphocyte populations. Their simulations suggest that an anti-cancer memory cytotoxic T lymphocyte pool of 3% or less can successfully eradicate a tumor cell population. Recently, Macfarlane *et al.* used a Lévy flight approach to capture the movement of inactive immune cells, Brownian motion to describe the movement of antigen-activated immune cells, and individual-based modeling to simulate the effects of immune cell activation, cancer cell proliferation, and the immune-induced destruction of cancer cells (Macfarlane, et al., 2018). Their simulation results reproduce spatial trajectories of immune cells observed in experimental data of single-cell tracking.

Systems biology projects that study exosome-mediated cancer-immune interactions are currently rare. Lu *et al.* developed the first kinetic model accounting for exosome exchange between tumor and immune cells (Lu, et al., 2014). The model was used to elucidate tumor-immune cell interactions and to identify potential therapeutic targets. It contains three variables representing tumor cells, antigen-presenting cells (e.g. dendritic cells) and tumor killer cells (e.g. T and B cells). The communication between these cell types is mediated by secreted cytokines and exosomes that carry molecular species such as ncRNAs (Figure 2 Left). Model simulations showed that the exosome exchange mechanism between tumor and dendritic cells provides a plausible explanation for the cancer immunoediting hypothesis. In this scenario, tumor cells can either be eliminated by, or escape from, a host immune attack depending on the features of the tumor microenvironment and on the genetic makeup of the tumor cells (Mittal, et al., 2014). Using their kinetic model, Lu *et al.* showed that these alternative fates for the tumor can be explained by the switch between three quasi-steady states of the model variable accounting for the tumor cell population (Figure 2 Right). Moreover, the authors used the model to evaluate the effectiveness of established cancer therapies (e.g. radiation, immunotherapy or both). Their model simulations showed that a potential effective therapy should not only reduce the size of the tumor, but also change the state of tumor cells in terms of the three quasi-steady states that were shown by the model simulations. While this model describes a general mechanism of exosome-mediated cancer-immune compartment interactions, the molecular basis accounting for the function of exosome-derived molecules, like lncRNAs, in regulating the immune response is not considered. In a recently published study, Friedman and Hao considered molecular functions of exosomal cargo. The authors developed a system of ordinary differential equations that includes variables for pancreatic cancer cells, immune cells, and exosomes, including two exosomal oncogenic miRNAs (Friedman and Hao, 2017). Model simulations showed that the exosomal miRNAs have the ability to facilitate tumor growth by inducing cancer cell proliferation and inhibiting T cell activation.

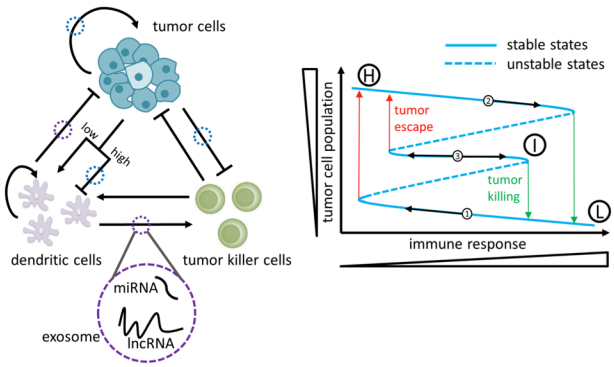


**Figure 2 Simplified kinetic model of tumor-immune compartment interactions. Left:** Scheme illustrating Lu's kinetic model (Lu, et al., 2014). The interplay between immune and tumor cells starts from the recognition of tumor-antigens by dendritic cells, and the intercellular communication further develops into the exchange of cytokines and/or exosomes (blue and purple circles). These exosomes may contain proteins but also specific ncRNAs such as miRNAs and lncRNAs. Cytokines and/or exosomes secreted by dendritic cells can suppress tumor growth or promote the proliferation and priming of tumor killer cells (e.g. NK and innate immune cells), while tumor-derived exosomes and/or cytokines can suppress immune activation by decreasing the proliferation of immune cells or increasing their apoptosis rates. Tumor killer cells can target and diminish tumor cell numbers and induce maturation of dendritic cells. Cancer and dendritic cells may establish autocrine loops by means of exosomes. **Right:** Illustration of the cancer immunoediting theory using model simulations. The bifurcation plot shows three stable quasi-steady states of the variable representing the size of the tumor cell population (tumor burden): high (H), intermediate (I) and low (L). At the onset of the tumor (①), if the immune cells fail to recognize the cancer cells, the population of tumor cells can leap from L to H (i.e. tumor escape). The sudden increase of tumor cells can activate the immune response (②), followed by transition from H to L by the immune cells (i.e. tumor killing). Due to the heterogeneity of tumor cell populations some tumor subpopulations are killed and others are retained at the primary site (i.e. the tumor cell population stays at I level; ③). With the development of cancer the retained cells can be recognized and removed by immune cells or escape from immune attack leading to tumor relapse.

## 5 Web resources for exosome cargo

With the discovery that EVs contain RNA molecules, sometimes referred to as evRNAs, and the advent of deep sequencing technologies a dramatic expansion in molecular data related to EVs and evRNAs could be observed. Consequently, several EV-specific databases have been developed that summarize the currently known components of EVs. ExoCarta for example, is a database that catalogues information from exosome studies (Keerthikumar, et al., 2016). For each study the mode of exosomal purification and characterization is provided as well as information about the biophysical and molecular properties of EVs. Data from ExoCarta or the investigator's own primary data on exosome content can be imported into FunRich a tool for performing functional enrichment (hypergeometric test) and interaction network analyses based on a collection of background databases (Pathan, et al., 2017).

miRandola is a manually curated database that is intended to classify extracellular circulating non-coding RNAs (Russo, et al., 2018). While initially focusing on circulating and exosomal miRNAs the database was

recently enriched with information on extracellular lncRNAs and circRNAs. miRandola is connected to miRó (Lagana, et al., 2009), the miRNA knowledge base, allowing users to infer the potential biological functions of circulating ncRNAs and their links to cellular phenotypes. The exoRBase database collects information about circRNAs, lncRNAs, and mRNAs in human blood exosomes based on RNA-seq data (Li, et al., 2018). In a coordinated effort of several academic societies/consortia including the International Society for Extracellular Vesicles, the Extracellular RNA Communication Consortium, and the American Society for Exosomes and Microvesicles a platform was established for the purpose of expanding the Gene Ontology (GO) in order to address the lack of standard terminology in the context of EVs (Cheung, et al., 2016). This research community defined extracellular RNA and EV-related terms and relationships and incorporated these into GO.

Most of the established gene signatures in exosomes and tumors are based on protein-coding genes because little was previously known about noncoding RNAs, especially lncRNAs, and their vital roles in cancer (Lanzós, et al., 2017). Many of the noncoding transcripts identified in next generation sequencing experiments have been annotated as *bona fide* lncRNAs. However, many questions about their expression and functions remain to be answered. This includes investigations into the functions of lncRNAs in cancers, their mechanisms and distribution.

Bioinformatics resources help to characterize tumor-related lncRNAs within the exosomal cargo. In this context, the GENCODE project serves as a major source for lncRNA annotations (Harrow, et al., 2012). Other lncRNA-related resources include NRED, lncRNAdb, NONCODE, LNCipedia, LncRNADisease, LncBase, and ChIPBase. These resources contain information about structure and genomic position of lncRNAs, their genomic and transcriptomic variants, functional characteristics, expression, interactions with other biomolecules (DNA, mRNA, miRNAs, and proteins) and their involvement in regulatory networks (Schmitz, et al., 2015). In addition, with the increasing interest in the regulatory functions of lncRNAs in complex human diseases such as cancer, there is a growing body of specialized databases that collate comprehensive and integrative data on the role of lncRNAs in human diseases. For example, the LincSNP database has integrated experimentally supported associations of single nucleotide polymorphisms (SNPs) in lncRNAs with disease and functional annotations of disease-associated lncRNAs (Ning, et al., 2016). Ning et al. have also developed the Lnc2Cancer database, which is an integrated resource for cancer-associated lncRNAs with experimental support that allows researchers to explore deregulated lncRNAs in various human cancers. The database contains 1,057 manually curated associations between 666 lncRNAs and 97 human cancers (Ning, et al., 2016). The lncRNASNP database provides information of SNPs and mutations in lncRNAs, as well as their impact on lncRNA structure and function (Miao, et al., 2018).

Current EV-related databases are summarized in Table 1 and 33 databases specialized on lncRNAs are listed in Supplementary Table 2.

**Table 1 Databases of exosomal cargo.**

| Database | Description | Web address | Reference |
|---|---|---|---|
| **ExoCarta** | A web-based compendium of exosomal cargo | www.exocarta.org | (Keerthikumar, et al., 2016) |
| **EVpedia** | An integrated proteome, transcriptome, and lipidome database of EVs | http://evpedia.info | (Kim, et al., 2015) |
| **Vesiclepedia** | A compendium for EVs with community annotations | www.microvesicles.org | (Kalra, et al., 2012) |
| **miRandola** | Manually curated classification of extracellular circulating miRNAs | http://mirandola.iit.cnr.it | (Russo, et al., 2018) |
| **exoRBase** | A database of circRNAs, lncRNAs, and mRNAs in blood exosomes | www.exoRBase.org | (Li, et al., 2018) |

## 6 Analysis of lncRNA-disease associations

Aberrant lncRNA expression or distorted interactions with other biomolecules can lead to or mediate cancer (Lanzós, et al., 2017). Algorithms for predicting lncRNA-disease associations can help to identify lncRNAs within the exosomal cargo that have previously not been associated with cancer. Current lncRNA-disease association prediction algorithms can be coarsely categorized into machine learning-based and network-based approaches. Most of these algorithms rely on previously identified lncRNA-disease associations for the inference of novel lncRNA-disease links. Some algorithms integrate transcriptomics data or employ functional similarity approaches to enhance their prediction performance.

Chen and co-authors have published a series of algorithms for the prediction of lncRNA-disease associations and lncRNA functional similarities. Their first algorithm, Laplacian Regularized Least Squares for LncRNA-Disease Association (LRLSLDA) in the semi-supervised learning framework, was developed based on their own previous work and that of others for miRNA disease association prediction (Chen and Yan, 2013). LRLSLDA incorporates lncRNA expression similarities (Spearman correlation) and Gaussian interaction profile kernel similarities for lncRNAs and diseases based on a known disease–lncRNA association network. A combined classifier determines the probability of a given lncRNA being related to a certain disease. Chen et al. enhanced the prediction performance of their algorithms by including various approaches for constructing lncRNA similarity networks (LNCSIM, ILNCSIM, FMLNCSIM) or developed complementary algorithms based on known lncRNA-disease associations (HyperGeometric distribution for LncRNA-Disease Association - HGLDA) or network-based models (KATZ measure for LncRNA-Disease Association prediction - KATZLDA) (see references in Supplementary Table 3).

The identification of cancer-specific lncRNA-disease associations is the aim of an algorithm developed by Zhao et al., which integrates multi-omics data to train a Naïve Bayes classifier. The algorithm predicted 707 novel putative lncRNAs linked to human cancers (Zhao, et al., 2015). A network-based model was employed by Liu et al. to predict cancer-related lncRNAs (Liu, et al., 2015). They applied Random Walk with Restart on a protein-coding gene/lncRNA bipartite network generated from prostate cancer transcriptomics and protein interaction data, and thereby identified 218 candidate lncRNAs putatively linked to prostate cancer. A network propagation algorithm was also applied in the work of Yang et al., in which a coding/non-coding gene–disease bipartite network was constructed based on known disease genes and lncRNA–disease associations (Yang, et al., 2014). The propagation algorithm, which is based on a semi-supervised learning approach, was used to uncover hidden lncRNA–disease associations in the bipartite network, which revealed 768 putative lncRNA-disease associations between 66 lncRNAs and 193 diseases. Some of their predicted lncRNA-disease

associations have been independently validated in pancreatic cancer and gastric cancer, among other diseases. Recently, Li et al. explored the functions of onco-lncRNAs across four solid cancers (bladder, prostate, lung, and breast) by analyzing co-expression networks using a Weighted Correlation Network Analysis (WGCNA) algorithm and identified 236 highly tissue-specific lncRNA-cancer associations (Li, et al., 2017).
A summary of 31 tools and algorithms for the prediction of lncRNAs, their function and putative disease associations is presented in Supplementary Table 3.

## 7 Conclusion

Accumulating evidence has shown that exosomes sustain tumor cells as carriers of autocrine, paracrine, and endocrine messages in the communication with the tumor microenvironment, the immune system, or even distal tissues. This form of intercellular communication can promote tumor cell aggressiveness and drug resistance, modulate adjacent cells in the microenvironment in favor of tumor growth, suppress immune response, and establish pre-metastatic niches (Figure 1). To better understand the signals that trigger these effects it is necessary to determine the composition of exosome cargo including lncRNAs (Supplementary Figure 1). Exosome analysis can be used as a novel strategy for cancer diagnosis and to monitor dynamic changes during cancer development and therapy. Circulating exosomal lncRNAs have an immense potential to refine current processes for diagnosis and prognosis of disease outcome. Abundant in human body fluids, they provide the opportunity for non- or minimally invasive rapid testing. They may also provide potential future therapeutic targets in the management of diseases.
Bioinformatics resources, focusing on features related to sequence, structure, and possible function of lncRNAs aid our understanding concerning the role of lncRNA in EVs and in intercellular communication. Systems biology approaches can help to model exosome-mediated intercellular communication and simulate effects triggered through perturbations to this communication system. Other computational methods, such as machine learning or network-based approaches can predict possible functions of lncRNAs and their potential involvement in diseases such as cancer.

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