Form and Function of Exosome-Associated Long Non-coding RNAs in Cancer

Chris Hewson and Kevin V. Morris

Abstract The recent discovery that long non-coding RNAs (lncRNAs) are functional and are not merely "transcriptional noise" has spawned an entirely new arena of investigation. LncRNAs have been found to be functional in the regulation of a wide variety of genes, including those involved in cancer. Studies have identified that lncRNAs play a role in the development and regulation of cancer and can also act as prognostic markers. Meanwhile, exosomes, which are extracellular particles generated endogenously by cells, have been observed to act as transport vesicles for a variety of biological components, particularly proteins and RNAs. This transportation of biological components has been shown to impact a variety of biological processes including the development of cancer. Collectively, these observations, along with those of several recent studies, suggest that lncRNAs and exosomes may function together to disseminate cell signals that alter and/or control local cellular microenvironments. This review will identify the various roles that lncRNAs and exosomes play in cancer development, as well as the possibility that exosomes may transfer functional lncRNAs between cells as a means of cell-to-cell communication.

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C. Hewson · K.V. Morris
Biotechnology and Biomedical Sciences, The University of New South Wales, Sydney, NSW 2052, Australia

K.V. Morris (✉)
Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA
e-mail: kmorris@UNSW.edu.au

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1 Long Non-coding RNAs

Through the human genome project, it was discovered that only 2 % of the human genome encodes for proteins; however, up to 90 % of the human genome is actively transcribed (Knowling and Morris 2011). These actively transcribed RNAs can be broken down into several different classifications including mRNA, tRNA, miRNA, siRNA and lncRNAs. Long non-coding RNAs are defined (somewhat arbitrarily) as transcripts greater than 200 nucleotides that do not code for proteins. They are generally not as highly conserved when compared to other types of RNA such as mRNA or miRNA (Struhl 2007). When lncRNAs were initially discovered, they were largely dismissed as "transcriptional noise" and thought to serve no particular function; however, further investigation has identified that lncRNAs are able to regulate gene expression using a variety of different mechanisms such as epigenetic regulation or transcriptional regulation (reviewed in Morris and Mattick 2014). LncRNAs have also been associated with various cancers as seen in the lncRNAs HOTAIR (Gupta et al. 2010) and MALAT1 (Gutschner et al. 2013), which opens up the possibility of targeting lncRNA-targeted cancer treatments or screening for them for use as biomarkers.

1.1 LncRNAs and X Chromosome Inactivation

It was originally thought that lncRNAs served no particular function and that they were merely excess transcripts produced during transcription (referred to as "transcriptional noise") (Struhl 2007). However, further investigation has disproved this notion and has shown that lncRNAs exhibit a variety of functions and have been shown to play a role in the epigenetic regulation of several genes (Kung et al. 2013). One example of this is during X chromosome inactivation. In females, one X chromosome is inactivated to ensure only one chromosome is expressed in each cell. In mammals, this is controlled by a cluster of lncRNA loci known as the X-inactivation centre (Brown et al. 1991). From this cluster of loci, a transcript known as the X-specific transcript is produced which is highly expressed during X

chromosome inactivation. This transcript coats the X chromosome in a "cloud" which acts as a scaffold to recruit silencing factors such as Polycomb repressive complex 2 (PRC2). This protein has the ability to methylate histones, primarily at histone 3 lysine 27 (H3K27). This results in the chromosome being remodelled into heterochromatin, thus preventing any transcription from that particular chromosome and ensuring that only one chromosome is actively transcribed. The X-specific transcript itself is also regulated by lncRNAs (Lee 2011).

1.2 LncRNAs Are Regulators of Tumour Suppressor Genes

Phosphatase and tensin homolog (PTEN) is a tumour suppressor protein, which is encoded by the PTEN gene, and it is frequently mutated in a large number of various cancers. For example, it has been demonstrated that in prostate cancer, up to 70 % of cancers have lost a copy of the PTEN gene (Chen et al. 2005). The PTEN gene was recently observed to be regulated by a PTEN pseudogene (PTENpg1), which has two isoforms, α and β. The α isoform of this pseudogene functions by recruiting DNA methyltransferase 3a (DNMT3a) as well as Enhancer of zeste homolog 2 (EZH2) which leads to the chromatin being remodelled resulting in lower expression of the PTEN protein (Johnsson et al. 2013), whereas the β isoform functions to increase PTEN expression by acting along with the PTENpg1 sense pseudogene as a miRNA sponge. This miRNA sponge binds to those miRNAs that are complementary to and/or targeted to the PTEN mRNA. Ultimately, the sponging of PTEN-targeted miRNAs by the PTENpg1 sense/antisense β isoform prevents the miRNAs from binding to the PTEN mRNA, which would normally lead to lowered expression of the PTEN protein (Johnsson et al. 2013).

Other lncRNAs, such as growth arrest-specific 5 (GAS5), have been shown to actively compete against other transcription factors. GAS5 functions by binding to the DNA-binding domain of the glucocorticoid receptor (GR), which prevents glucocorticoid response elements (GRE) from binding to the GR. This affects the transcription of target genes which includes a variety of apoptosis inhibitors such as the cellular inhibition of apoptosis protein 2 gene (cIAP2) (Kino et al. 2010). By preventing the transcription of these apoptosis-inhibiting genes, GAS5 has the capability to make cancer cells susceptible to apoptosis. It has been demonstrated that in prostate cancer cell lines, high levels of GAS5 caused the cells to be far more susceptible to chemotherapeutic agents and radiation (Pickard et al. 2013). This observation suggests that GAS5 could possibly be explored as a possible avenue of cancer treatment, particularly in cases where the cancer is resistant to chemotherapy and radiation. Furthermore, GAS5 expression is very low in several cancer cell lines (such as breast and leukaemia cancer cells), while in normal cells, the expression is much higher. These observations suggest that GAS5 may play a functional role as a tumour suppressor (Mourtada-Maarabouni et al. 2009).

The lncRNA maternally expressed gene 3 (MEG3) is an example of another lncRNA that exhibits tumour-suppressing capabilities. MEG3 functions by

stimulating P53 expression and can also inhibit cell proliferation independent of the p53 protein (Zhou et al. 2007; Zhang et al. 2010). Normally, p53 protein levels are kept low due to its constant degradation via the ubiquitin–proteasome pathway, which is regulated by the mouse double minute 2 homolog (MDM2) gene. MEG3 functions by inhibiting MDM2 expression, thus preventing p53 from being ubiquinated and resulting in higher levels of the p53 protein (Benetatos et al. 2011). The knockout of MEG3 results in the increased expression of vascular endothelial growth factor signalling genes (Gordon et al. 2010). These observations imply that MEG3 inhibits angiogenesis and may have multiple methods of acting as a tumour suppressor. MEG3 is also capable of binding to the PRC2 suggesting that MEG3 may also be able to regulate gene expression via the structural modification of chromatin (Zhao et al. 2010).

1.3 LncRNAs Have the Ability to Promote Cancer

LncRNAs have also been observed to affect the gene expression of tumour-related genes by binding to proteins such as transcription factors. This mechanism is demonstrated by the lncRNA P21-associated ncRNA DNA damage activated (PANDA) which is found slightly upstream of the CDKN1A/p21 locus. PANDA has been observed to exhibit changes in expression in response to DNA damage and activation of the p53 gene (Morachis et al. 2010). PANDA interacts with the transcription factor NF-YA which prevents it from binding to pro-apoptotic genes such as FAS or BIK (Hung et al. 2011). Without the binding of the transcription factor, the pro-apoptotic genes are prevented from being expressed and can cause an increased survival rate within cancer cells.

Studies have observed that lncRNAs can play a variety of different rolls within cancers and can influence tumour metastasis as well as tumour suppression. This is seen in the HOX transcript antisense RNA (HOTAIR). HOTAIR was observed to impact on tumorigenesis and exhibited up to a 2000-fold increase in expression in breast cancer cells relative to normal human breast epithelia (Gupta et al. 2010). This high expression was discovered to be a significant indicator of tumour metastasis and poor patient prognosis. It was also shown that silencing the HOTAIR lncRNA using RNA interference leads to both decreased cell viability and lower cell metastasis, suggesting that particular lncRNAs can have a dramatic impact on the characteristics of cancer cells (Gupta et al. 2010). HOTAIR also functions by epigenetically regulating gene expression via the recruitment of chromatin-modifying complexes such as PRC2 and LSD1. HOTAIR acts as a scaffold for these chromatin-modifying complexes as the 5' end binds to PRC2, while the 3' end binds to LSD1 which leads to chromatin modification and ultimately a change in gene expression via H3K27 methylation and lysine 4 demethylation (Tsai et al. 2010).

Another cancer-related lncRNA is metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) which is expressed endogenously in most human tissue but has been found to be up-regulated in several types of human cancers such as breast

(Guffanti et al. 2009), prostate (Lin et al. 2006) and liver cancer (Lin et al. 2006). MALAT1 was identified as an oncogene that promotes tumorigenesis and therefore is also associated with a high chance of metastasis and a poor patient prognosis (Guffanti et al. 2009). Knockdown of MALAT1 leads to a wide array of phenotypes including the inhibition of angiogenesis, cell cycle progression (Michalik et al. 2014), cell mobility (Tano et al. 2010) and a higher incidence of cell death (Tripathi et al. 2010). MALAT1 functions by regulating the alternative splicing of endogenous target genes (Tripathi et al. 2010). It has also been suggested that MALAT1 may interact with PRC2 to regulate genes epigenetically (Guil et al. 2012). These observations further highlight the importance of the role that lncRNAs play in the regulation of cancer cells.

1.4 Telomere Length Is also Regulated by LncRNAs

Telomere length is another cellular function that has been demonstrated to be regulated by the action of a lncRNA. Telomeres are regions of repeated nucleotide sequences found at the ends of chromosomes, and they act as a barrier to prevent chromosomes from degrading or fusing with each other. However, due to the mechanisms of DNA replication, each time the cell divides and the DNA is replicated, a small fraction of the telomere is lost and the telomere region ultimately becomes shortened. Eventually, after numerous DNA replications, fragments of essential genes are lost which may result in the eventual death of the cell. Cell immortality and limitless replicative potential is one of the hallmarks of cancer, and for this to be achieved, cancer cells must solve the problem of shortening telomeres. This is remedied by the enzyme telomerase, which has the ability to lengthen telomeres. Recent observations have suggested that the lncRNA, TERRA, plays a role in regulating telomerase function. Studies have demonstrated that TERRA inhibits telomerase activity suggesting that it may regulate telomere length negatively. Knockdown of the lncRNA, TERRA, results in shorter telomere length (Redon et al. 2010), and TERRA expression has also been found to be down-regulated in many cancer cells (Schoeftner and Blasco 2008) which further supports this hypothesis. Although the exact functional mechanism behind TERRA is still not well understood, it has been postulated that TERRA may bind to telomere regions to prevent the binding of telomerase. Another hypothesis is that TERRA binds to telomerase itself which causes a conformational change and prevents telomerase from functioning normally (Redon et al. 2010).

2 Exosomes

Exosomes are small membrane vesicles (40–100 nm) with a saucer-like morphology resembling flattened spheres that are endogenously released from cells into the intracellular environment. Exosomes were first observed in mammalian

reticulocytes (immature red blood cells) and found to be released during reticulocyte maturation to erythrocytes. Similar to lncRNAs, exosomes at the time of their discovery were also thought to have no biological significance (Johnstone et al. 1987; Johnstone 2006). The term "exosomes" has previously been used loosely and interchangeably to describe microvesicles. Microvesicles are much larger (up to 1 μm) and are generated via a completely different pathway relative to exosomes. Microvesicles are released when the plasma membrane is shed directly into the extracellular space. Meanwhile, exosomes are secreted when specific endosomal compartments known as multivesicular bodies (MVBs) fuse with the plasma membrane. It is also important to note that the cell is capable of secreting both exosomes and microvesicles simultaneously which can often make the isolation of pure exosomes difficult to impossible (Lee et al. 2011). Exosomes contain a large variety of biological components such as proteins, mRNAs, miRNAs (Rani et al. 2011) and lncRNAs (Spizzo et al. 2012). Since their discovery, exosomes have been demonstrated to impact various biological functions such as cell communication, the immune system and tumour metastasis (Li et al. 2006). Exosomes also have the ability to cross the blood–brain barrier without eliciting an immune response suggesting that they could prove exceedingly useful in developing brain-targeted drug delivery systems (Alvarez-Erviti et al. 2011).

2.1 Exosome Biogenesis

Exosomes originate as MVBs that are found within the endocytic cycle of a cell. MVBs are a type of endosome, which are characterized by containing several membrane-bound intraluminal vesicles (ILVs). These vesicles are formed via a direct budding into the lumen of a MVB (Klumperman and Raposo 2014). The formation of these MVBs is achieved by the endosomal sorting complexes required for transport (ESCRT). The ESCRT complex consists of ESCRT-0, I, II and III. ESCRT-0, I and II aid the generation of MVBs by clustering ubiquitinated proteins and then binding them to the membrane of an endosome. The proteins are then absorbed into the endosome via a direct budding into the lumen to form an ILV. ESCRT-III forms a ring-like structure around the vesicles where the proteins are absorbed into the endosome. This prevents any proteins from leaking out into the cytoplasm during the formation of the ILV. Once the proteins have been transferred to the MVB via the ILV, the Vps4-Vta1 proteins remove the ESCRT components from the endosomal membrane, thus completing generation of the MVB (Raiborg and Stenmark 2009; Henne et al. 2011). Silencing of the ESCRT complex has been shown to result in a decrease in the number of exosomes which are secreted by the cell highlighting their importance in exosomes formation (Colombo et al. 2013).

After the formation of the MVB, it needs to be transported to the cellular membrane before it can be secreted as an exosome. While the exact mechanism behind this process is still not well understood, it has been postulated that in order to transport the MVBs to the cell membrane, a combination of cellular framework

such as actin and microtubules along with molecular motors including myosins and kinesins may be utilized (Cai et al. 2007). Once the MVB has reached the cell membrane, it must fuse with the membrane before it is released as an exosome. A family of membrane proteins known as SNAREs mediates this fusion event. A SNARE protein binds to the MVB (v-SNARE), while another one binds to the cell membrane (t-SNARE). The two proteins then bind to each other to fuse the MVB to the cell membrane (Cai et al. 2007). Notably, the Golgi apparatus creates endosomes which can be altered into MVBs. These MVBs can be degraded by the lysosomes or be secreted by the cell as exosomes. Exosomes taken up by recipient cells can also be fused into MVEs and recycled out of the cell, or they can be degraded by the lysosome.

After the MVB is bound to the cell membrane, it can then be ejected from the cell as an exosome. The release of exosomes involves the recruitment of several Rab proteins (family of proteins which belong to the Ras superfamily). Rab proteins bind with the cellular membrane to regulate vesicle budding, vesicle transport and membrane fusion. The proteins Rab27a and Rab27b seem to play pivotal roles in exosome secretion, as the suppression of these proteins results in a reduced number of exosomes released from the cell (Ostrowski et al. 2010). Rab27 has also been found to be associated with the secretion of other organelles from the endocytic pathway, further solidifying the hypothesis that they play a crucial role in the secretion of exosomes (Raposo et al. 2007).

How exosomes are targeted to be taken up by recipient cells remains unknown. It is understood that the binding of exosomes to cell membranes is controlled by cell adhesion molecules used in cell-to-cell interactions such as integrins and intercellular adhesion molecules (ICAM). In order for the exosome to deliver its contents, they are absorbed by the cell and undergo endocytosis. Once the exosome is broken down by the lysosome, its contents can be released into the cell where they may have functional relevance (Record et al. 2014). In this manner, it may be that exosomes are generalized in their targeting of recipient cells, though this notion has not been thoroughly vetted experimentally.

2.2 Exosome Function

It has been hypothesized that exosomes may function as a form of cell-to-cell communication. Due to the fact that proteins and RNA are prone to degradation in the extracellular space, one postulated function for exosomes is that they protect biological compounds from degradation during travel between cells in the extracellular space. Notably, many of the documented exosomal proteins and RNAs have been observed to be functional once absorbed by recipient cells (Valadi et al. 2007). This further solidifies the notion that exosomes are a method of communication utilized by cells to communicate with each other in between the extracellular space (depicted in Fig. 1).

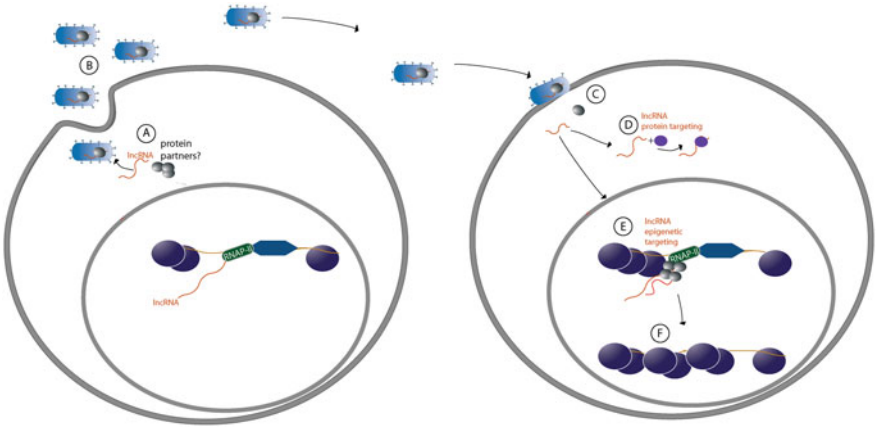

Fig. 1 Exosome-mediated delivery of lncRNAs to target cells. A schematic is shown depicting a generalized model for the spread of lncRNAs from one cell to another via the action of exosomes. *A* The lncRNA may interact with exosome packaging proteins resulting in *B* the release of the exosomes containing candidate lncRNAs. *C* The lncRNA-containing exosomes can then bind and internalize into recipient cells. The lncRNA may then *D* target cellular proteins to affect function or *E* target homology-containing genes and modulate transcription which could lead to *F* stable epigenetic silencing of the lncRNA-targeted gene

2.3 Exosomes Transferring Chemoresistance Between Cells

Resistance to traditional methods of cancer treatments such as chemotherapy or radiation remains to be one of the major hurdles when treating cancer. In order to overcome these hurdles, a more complete understanding of the mechanisms, which allow for this resistance, is required. Many studies have demonstrated that exosomes are capable of playing a key role in regard to cancerous cells obtaining this trait. An example of this is seen through the secretion of Survivin via exosomes. It has been demonstrated that uptake of Survivin via exosomes protects the cell from radiation damage by promoting cell proliferation and improving metastatic potential (Khan et al. 2009). Another study observed that prostate cancer cells which were previously susceptible to docetaxel could obtain resistance to this drug via the exosomal transfer of multidrug resistance protein 1 (MDR-1), a drug transporting glycoprotein which has the ability to pump docetaxel out of the cell. A similar mechanism was observed in the breast cancer cell line MCF-7. MCF-7 cell lines which were previously sensitive to chemotherapeutic agents could inherit chemoresistance via exosomes which originated from drug-resistant variants of the MCF-7 cancer cell line. This occurred due to the miRNAs within the exosomes which were found to knock down various genes such as the important tumour suppressor gene PTEN (Chen et al. 2014).

2.4 Exosomes Impacting Tumorigenesis

Exosomes derived from cancer cells have also been shown to promote tumour invasion and metastasis. Exosomes isolated from highly metastatic variants of melanoma possess the ability to increase the metastatic behaviour of primary tumours via the receptor tyrosine kinase MET (Peinado et al. 2012). Further observations have shown that breast cancer exosomes contain miRNAs as well as the machinery to process precursor miRNAs into mature, functioning miRNAs (such as Dicer, TRBP and Ago2). This leads to a reprogrammed transcriptome, which can induce tumour formation within non-tumourigenic epithelial cells. The inhibition of dicer function within exosomes also demonstrated impaired tumour growth within recipient cells, thus identifying the importance of miRNA processing in regard to exosomes promoting tumorigenesis (Melo et al. 2014).

Previous studies have observed that exosomes can facilitate the transfer of oncoproteins, such as mutant KRAS, between cells to induce tumourigenesis. KRAS is a signalling protein that is essential in many cell-signalling pathways. A mutation in the KRAS gene is a common step in the development of several cancers (Kranenburg 2005). Exosomes originating from colon cancer cells possess the ability to transfer mutant KRAS between cells, resulting in stimulated cell growth, thus increasing the recipient cells chances of becoming tumorigenic (Demory Beckler et al. 2013). Exosomes are also capable of promoting angiogenesis within recipient cells. Glioblastoma cells release exosomes, which contain mRNA, miRNA and proteins that promote angiogenesis. Observations have demonstrated that when these exosomes were taken up by recipient endothelial cells, angiogenesis was stimulated (Skog et al. 2008). Collectively, these observations demonstrate how cancerous cells can utilize exosomes as a method of cell communication in order to induce cancer-like characteristics in healthy cells.

2.5 Exosomes Influencing the Immune System

Exosomes released from cancer cells possess the ability to impact the immune system in order to aid tumour proliferation. Exosomes isolated from lung cancer cells contain miRNAs which prevent toll-like receptors (TLRs) from being expressed in macrophages. The miRNAs bind to the TLR mRNA, which leads to their respective degradation. This results in an increased secretion of pro-inflammatory cytokines from the macrophage and causes tumour cells to spread throughout the body (Fabbri et al. 2012). Another study demonstrated that colorectal cancer exosomes contained Fas ligand, tumour necrosis factor and various other proteins involved in the induction of apoptosis. These exosomes were taken up by recipient T cell initiating apoptosis and death of the recipient cell. This prevents T cells from destroying cancerous cells and ultimately permits tumour proliferation (Huber et al. 2005).

2.6 *Exosomes as a Possible Method of Cancer Treatment*

Exosomes are currently being explored as a possible tool to treat cancers and a variety of other diseases. Current research is attempting to utilize exosomes as a method of drug delivery as they do not elicit an immune response and are capable of crossing the blood–brain barrier. This was recognized when exosomes derived from dendritic cells within mice were engineered to express Lamp2b, a membrane protein which fuses to neurons. These exosomes were then loaded with a siRNA to knock down GAPDH via electroporation before being injected into the mice. A brain-specific knockdown of GAPDH was observed within the mice as well as a lack of an immune response (Alvarez-Erviti et al. 2011).

The realization that exosomes may have therapeutic use first arose when it was observed that dendritic cells were capable of secreting antigen-presenting exosomes which possessed functional MHC class I and II molecules. These exosomes were able to initiate cytotoxic T cells which resulted in the suppression of tumour growth in vivo within mouse models (Zitvogel et al. 1998). Further investigation demonstrated that these exosomes released from dendritic cells contained heat-shock cognate protein hsc73. This protein is considered to be a key factor in inducing immune responses against cancer cells (Thery et al. 1999). A similar anti-cancer effect was observed from exosomes secreted by endothelial cells. The endothelial cells released exosomes which contained the miRNA miR-503 which when taken up inhibited the proliferation and invasiveness of the breast cancer cell line.

The impact of heat-shock proteins (hsps) in cancer and exosomes has recently been identified in several studies. Hsps are a family of proteins which are activated when the cell is exposed to stressful conditions such as high temperatures (Åkerfelt et al. 2010). Hsps play an important role in the immune system as they bind to antigens and are involved in antigen presentation T cells. Due to this characteristic, hsps are being tested for use as possible immunological adjuvants within vaccines, including cancer vaccines (Bolhassani and Rafati 2008). Previous studies have identified that when B lymphoblastoid cells were exposed to a 42 °C heat shock, the exosomes released by the cells contained higher levels hsps relative to the control (Clayton et al. 2005). A similar effect was observed when a hepatocellular carcinoma cell line was treated with various anti-cancer drugs. It was identified that the drug treatment resulted in an increased level of hsps within the exosomes which were secreted from the treated cancer cells (Lv et al. 2012). Exosomes enriched with hsps have initiated anti-tumour immune responses leading to tumour regression within murine models. These examples identify the importance of heat-shock proteins and exosomes as a possible method of cancer treatment (Cho et al. 2009).

A previous study observed that the tumour suppressor protein PTEN is secreted by cells via exosomes. PTEN functions by regulating the PI3K–AKT pathway which is an essential pathway in cell cycle regulation and if mismanaged can result in high levels of cellular proliferation which can often result in cancer (Vanhaesebroeck et al. 2012). The PTEN protein within the exosomes has been identified as functional when taken up by neighbouring cells, thus ensuring that the

cell proliferation is adequately regulated and preventing any tumours from developing (Putz et al. 2012).

Exosomes are currently undergoing clinical trials within humans for a variety of different cancers. Exosomes containing MAGE 3 peptides were introduced to stage III/IV melanoma patients in an attempt to immunize them. Patients displayed minimal side effects after treatment, and it was demonstrated that exosomes had minimal toxic effects. This study has also served as a proof of concept that exosomes can be produced at a large scale for human therapeutics. This body of work has spawned multiple exosome-based cancer therapies that are currently undergoing clinical evaluation. For example, NCT01159288 is currently undergoing a phase II clinical trial and utilizes exosomes derived from dendritic cells which are loaded with tumour antigens as a vaccine against advanced non-small cell lung cancer. Meanwhile, NCT01344109 is undergoing a pilot study, which involves using exosomes secreted from tumours as diagnostic and prognostic marker for patients undergoing chemotherapy. NCT01779583 is a similar clinical trial where exosomes are being used as a prognostic marker for gastric cancer. While these clinical trials are indicative of the potential use of exosomes as cancer therapeutics, they are merely scratching the surface of their potential. Eventually, human engineered exosomes containing drugs or therapeutic biologicals may enter clinical trials and their full potential may be realized.

2.7 Exosomes and LncRNAs

Recent observations suggest that exosomes may act as transport vesicles for functional lncRNAs which may result in a phenotypic effect within the recipient cell (Kogure et al. 2013) (Fig. 1). The lncRNA TUC339 was identified in exosomes derived from hepatocellular carcinoma (HCC) and shown to be highly expressed in exosomes. Suppression of this lncRNA in cells using RNA interference leads to reduced cell proliferation, clonogenic growth and cellular adhesion (Kogure et al. 2013). This observation suggested that cells utilize exosomes and TUC339 in an attempt to increase cell proliferation of nearby cells. ROR is another lncRNA that was found to be highly overexpressed in exosomes derived from HCC cells treated with doxorubicin. HCC cells were treated with exosomes containing high levels of ROR, and an increased level of chemoresistance was observed. Similarly, knockdown ROR within HCC cells using RNA interference leads to increased sensitivity to chemotherapeutic agents (Takahashi et al. 2014). This implies that cancerous cells may be utilizing lncRNAs and exosomes to improve chemoresistance within neighbouring cells.

Several previously described lncRNAs such as MALAT1, HOTAIR and GAS5 have also been found to be highly expressed within exosomes from HeLa and MCF-7 cells. These previously described lncRNAs play important roles within a variety of cancers suggesting that the cancerous cells are releasing these exosomes to try and induce cancer-like phenotypes within the recipient cells (Gezer et al. 2014).

This also suggests that these lncRNAs are being selectively packaged into exosomes; however, the mechanism behind packaging specific biological contents into exosomes is not well understood at this time. Another possible yet to be explored function of exosome-associated lncRNAs may be to deliver lncRNAs that are capable of directing epigenetic silencing (Fig. 1). While not yet reported for exosome-associated lncRNAs, there are several known lncRNAs capable of controlling transcriptional and epigenetic states (Morris and Mattick 2014). Collectively, the observations presented to date support the notion that exosomes may function as transport elements for lncRNAs and possibly function in cell-to-cell communication.

3 Conclusion

Although once considered transcriptional noise, recent research has identified that lncRNAs play a functional role in gene expression, regulation and cancer (Morris and Mattick 2014). Furthermore, unlike other RNAs such as miRNAs, lncRNAs seem to have several mechanisms or modes of action by which they function. This includes binding to chromatin to promote epigenetic regulation, acting as scaffolds for proteins and acting as a miRNA sponge just to name a few reported functions. Their functional relevance in cancer also presents the possibility of using lncRNAs as diagnostic or prognostic markers as well as cancer therapeutic targets. Exosomes have also been demonstrated to be impactful on cancer and cellular function via the transfer of biological components. The previous examples indicate that they promote tumour-like characteristics, impact the immune system and induce chemoresistance within cells. Indeed, exosomes are currently being assessed as a possible method of targeted drug delivery and are currently undergoing multiple clinical trials. Studies have also provided examples of lncRNAs and exosomes functioning together to control gene expression and cell phenotypes within nearby cells. Considering that most exosome research has focussed on miRNA and proteins, this opens up an interesting new area of research in investigating the functions of lncRNAs within exosomes and those cells targeted by the lncRNA-containing exosomes.

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