Cancer exosomes as microRNA factories

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Abstract

MicroRNAs modulate gene expression while exosomes are extracellular cargo vessels which transport microRNAs and other materials to surrounding cells. When exosomes are taken up by recipient cells, the released miRNAs can modulate immune responses, inhibit apoptosis and promote angiogenesis in order to maintain tumour growth. Central to this regulation is the processing of the primary transcripts into active miRNAs that occurs exclusively within mammalian cells. Challenging this dogma, is the discovery that Dicer and Ago2, key components of miRNA processing, are also inside exosomes. While the exact nature of this processing requires extensive proof, it is an exciting notion that exogenous miRNA factories could exist outside the boundaries of the traditional mammalian cell.

Why are miRNAs important in cancer?

The discovery of the small non-coding RNA (ncRNA) family such as microRNAs (miRNAs, miRs) has profoundly reshaped our understanding of gene regulation in both normal and disease conditions. These miRNAs are potent 22 nucleotide RNA strands which control gene expression at both the translational and post-transcriptional interface. The majority of miRNAs directly bind to the 3' untranslated region (UTR) of the target mRNA and abate any further translational progression of the nascent message. Using this modality of gene regulation, miRNAs can suppress the expression of classical tumour suppressors or promote expression of oncogenes to drive various oncogenic processes [1]. Beyond this role, they themselves can also take on the function as either OncomiRs or Tumour Suppressor miRs. The in vivo overexpression of specific oncomiRs such as miR-21 could induce a phenotype resembling pre-B cell lymphoma [2]. In contrast, the administration of intranasal let-7 was able to reduce the growth of lung cancer xenografts in immuno-deficient mice [3].

There are approximately 2588 human miRNAs deposited in miRBase version 21 [4] but only 585 of these have been documented to have an association with 90 or more different cancer types (Supplementary Table 1. [5])These include solid tumours such as breast, stomach, prostate, liver, head and neck and blood cancers encompassing lymphomas and leukaemia. This association extends to both adult and childhood cancers [6]. miRNAs can also play a central role in the epithelia-mesenchymal-transition (EMT); a transformation process driving the early phase of cancer metastasis [7]. Within the tumour microenvironment, these ncRNAs can regulate both the tumour and surrounding stroma cells to drive tumour angiogenesis, immune invasion and the reprogramming of fibroblasts into cancer associated fibroblasts. Given their regulatory dominion over so many oncogenic processes, this has driven the

clinical development for using miRNAs as novel drug targets and biomarkers for cancer diagnosis.

Exosomes and miRNA processing

The biogenesis of mammalian miRNAs begins in the nucleus with transcription of a typical mRNA which harbors specific miRNAs. This primary RNA transcript is enzymatically cleaved by Drosha (Figure 1), followed by a second cleavage event mediated by Dicer to generate the short double stranded mature miRNA. Lastly, this duplex is loaded onto Ago2, but only one strand of the duplex is incorporated for direct binding to the target RNA [8].

The current dogma is that most of this biogenesis only occurs inside mammalian cells with miRNA mediated regulation mostly contained within the cytoplasm. This belief is now being challenged by the discovery of miRNAs and their machinery inside cancer exosomes.

Exosomes are secreted membrane vesicles typically ranging in size from 40-100nm, and they function as transports vehicles harboring, DNA, RNA, lncRNAs, miRNAs and protein. They are formed through the multivesicular endosomes pathway and deliver their cargo to both surrounding cells and can travel to distal parts of the body. It is now widely accepted that exosomes operate as extracellular vehicles for intercellular communication between cells. The first report of miRNAs inside exosomes suggested that exogenous gene regulation by miRNAs was indeed possible [9]. Since this landmark discovery, there has been a deluge of studies investigating the novel role of exosomal miRNAs in cancer. We now understand that tumour cells secrete greater quantities of exosomes into the vascular system and this miRNA cargo can regulate immune evasion, inhibition of apoptosis and angiogenesis to promote

tumour growth. Added, the vasculature can rapidly traffic the miRNA cargo to distal cells to cultivate the surrounding cellular landscape to create a pre-metastatic bed.

Beyond their capacity as merely miRNA cargo containers, exosomes may potentially carry out miRNA biogenesis independent of the canonical pathway found in mammalian cells (Figure 1). Exosomes isolated from breast cancer cells and incubated in serum free media showed an increased abundance of mature miRNAs and decreased abundance of precursor miRNAs. Dicer, Ago2, TRBP, and RISC-loading proteins were detected in these exosomes, which supported the idea that miRNA factories can exist inside cancer exosomes. Notably, there was no accumulation of the same mature miRNAs or the presence of any biogenesis machinery in normal breast exosomes [10]. It was also shown that, breast cancer cells could shuttle excess Dicer and Ago2 into their exosomes, whereas this transport was not witnessed in normal cells. In a recent study using colorectal cancer cells, Ago2 sorting into exosomes was mediated by the mitogen-activated protein kinases (MEKs) which are part of the activated KRAS signaling pathway. Furthermore, the phosphorylation of Ago2 on S387 re-diverts Ago2 from cancer exosomes into distinct cytoplasmic foci known as the Processing-bodies [11]. The active shuttling of Ago2 and perhaps other biogenic components may support the capacity for exosomes to carry out exogenous miRNA processing. These findings have ignited the field both in excitement and controversy. Notwithstanding the latter, other studies have definitively shown the presence of Dicer, Ago2 and GW182 residing inside exosomes[11-13].

In a very elegant study, it was concluded that cancer exosomes on average, contained only a single miRNA per exosome. However, it is likely that cancer exosomes released from different cancer cells have varying stoichiometric levels of specific miRNAs [14]. Perhaps

processing is restricted to only exosomes containing high levels of certain miRNAs. If we extend this notion to examine the stoichiometric levels of both cytoplasmic Ago2 and Dicer, their endogenous levels may not be able to cope with the sudden deluge of exosomal precursor and mature miRNAs into recipient cells. To deal with this sudden stoichiometric rise in these ncRNAs, exogenous miRNA processing may take place to advert saturation of the endogenous miRNA machinery.

We have detected primary-miR-21 and others inside cancer exosomes but we do not know if they undergo processing to generate mature miRNAs. The likely outcomes are that, upon exosome uptake, these primary miRNAs are processed by Drosha in the nucleus of the recipient cell, or the exosomes are mini factories for miRNA processing. Alternatively, these primary miRNAs may not be processed at all.

Concluding Remarks

Inside cancer exosome we can find several key components of the miRNA processing pathway along with the primary, precursor and mature miRNAs strands. The combination of these pieces increase the plausibility that cancer exosomes can mediated exogenous processing. If this is valid, cancer exosomes could become autonomous bodies capable of processing primary and precursor miRNAs into the active mature miRNA ready for modulation of cancer related pathways. The challenge for this field is to consolidate and reproduce these initial findings. This would include a clear demonstration showing the complete processing of a primary miRNA into a mature miRNA. It is also unclear if only specific miRNAs families are shuttled into exosomes for processing and what other biogenic proteins besides Ago2 are actively moved into cancer exosomes.

If these observations are proven valid, the disruption of exogenous processing or inhibiting Ago2 traffic and others into cancer exosomes could eliminate or dampen the oncogenic effects of these miRNAs. However, before we get too excited, this is an emerging area of research with few studies which must now be validated by other teams before the premise of mini processing factories can be accepted as common dogma.

Figure Legends.

Figure 1. The canonical miRNA biogenesis pathway in mammalian cells. Transcription and processing begins in nucleus and is completed in the cytoplasm with the generation of 22 nucleotide RNA duplex. In the nucleus RNA Pol II generates a primary miRNA transcript which harbors the mature miRNA. This primary miRNA is cleaved by the RNASE III enzyme, Drosha to form the intermediate precursor miRNA. This stem loop structure is exported to the cytoplasm wherein Dicer mediates a second cleavage event to generate the 22 nt miRNA duplex. Only one strand of the miRNA duplex is loaded onto Ago2 and this complex is now able to recruit other accessory proteins to mediate target gene silencing. In this model, it may be possible that, the biogenesis machinery, such as Dicer and Ago2, are also shuttled into the exosomes along with primary and precursor miRNAs. The mitogen-activated protein kinases and perhaps Alix can potentially regulate the the shuttling of Ago2 into cancer exosomes. The mechanism for Dicer transport into these exosome is not fully known. Upon packaging of these miRNA components and other bioactive materials (proteins, DNA and mRNA), these cancer exosomes are release into the extracellular space.

Table 1: This is a summary of the known association between exosomes and various cancers. The list was compiled from the miRCancer database (http://mircancer.ecu.edu) detailing more than 3700 published studies which show that over 90 cancers have a association with the aberrant expression of miRNAs.

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