Seminars in Cancer Biology 27 (2014) 46–51

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Disruption of microRNA nuclear transport in human cancer

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ARTICLE INFO

Keywords:
Exportin-5
Pre-miRNAs
Microrna biogenesis
miRNA biogenesis impairment
Nuclear export pre-miRNAs

ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally. MicroRNAs target about 80% of the protein-coding mRNAs and therefore can be considered master regulators of multiple cellular pathways, contributing to the fine-tuning the cell's most important processes, like the ones involved in cellular growth and proliferation. Deregulation of miRNAs plays a fundamental role in the onset, progression and dissemination of many cancers; therefore impairment of miRNA biosynthesis is an important event in the tumorigenic cascade. MicroRNA synthesis is a multistep regulated process that requires transport of RNA molecules from the nucleus to the cytoplasm. The immature miRNA species that are produced in the nucleus are exported through the nuclear pore complexes *via* mobile export receptors. Small RNAs such as precursors of miRNAs (pre-miRNAs) are transported out of the nucleus by a specific nuclear transport receptor, exportin-5 (XPO5). Pre-miRNA nuclear export is a fundamental step in miRNAs biosynthesis and its deregulation through inactivating mutations in the XPO5 gene can lead to pre-miRNA nuclear accumulation and disturbance of mature miRNA expression. In addition, it is becoming increasingly evident that mature miRNAs also function as gene regulators in the nuclear compartment. In this review, we will discuss the export of miRNA precursors and its impairment in human cancer as well as the recently described nuclear functions of miRNAs.

1. MicroRNAs biogenesis

MicroRNA-guided mRNA silencing is a post-transcriptional gene regulatory process based on a family of endogenous non-coding RNAs of ~19–24 nucleotides called microRNAs (miRNAs) [1]. Repression of mRNA targets leads to decreased translational efficiency and/or decreased mRNA levels [2]. The main biological function of miRNAs is mediated through recognition of specific binding sites of imperfect complementarity usually located in the 3′ untranslated region (UTR) of mRNAs [3]. In order to use miRNAs to down-regulate mRNA targets, cells must first process these ~22 nt RNAs from primary transcripts (pri-miRNAs), a process that occurs in two sequential regulated steps in the nucleus and cytoplasm of the cell (Fig. 1). Pri-miRNAs are transcribed by RNA polymerase II (RNAPII) from specific transcripts of independent genes or from introns of protein-coding genes [1,4]. In the canonical pathway, pri-miRNA processing occurs in two steps, catalyzed by two members of the RNase II family of enzymes, Drosha and Dicer, operating in complexes with dsRNA-binding proteins (dsRBPs), DiGeorge Syndrome Critical Region Gene 8 (DGCR8) and transactivation-responsive RNA-binding protein (TRBP) in mammals [5,6]. In the first step, the stem loop of the pri-miRNA is recognized by the nuclear Drosha-DGCR8 complex that processes into a ~70-nucleotide precursor hairpin (pre-miRNA), which is exported to the cytoplasm [6]. The hairpin-shaped pre-miRNA is then transported from the nucleus to the cytoplasm by exportin-5 (XPO5), where it is loaded by the Dicer–TRBP complex and cleaved into a double-stranded miRNA in a process known as dicing [6]. Some pre-miRNAs are produced from very short introns (mirtons) as a result of splicing and debranching, thereby bypassing the Drosha–DGCR8 step. In either case, cleavage by Dicer, assisted by TRBP, in the cytoplasm yields an ~20-bp miRNA/miRNA* duplex. It has been recently shown that human Dicer has the ability to shuttle between the nucleus and the cytoplasm but the protein steady-state localization is cytoplasmic [7]. After strand separation, the mature miRNAs, in combination with Argonaute proteins, form

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http://dx.doi.org/10.1016/j.semcancer.2014.02.012
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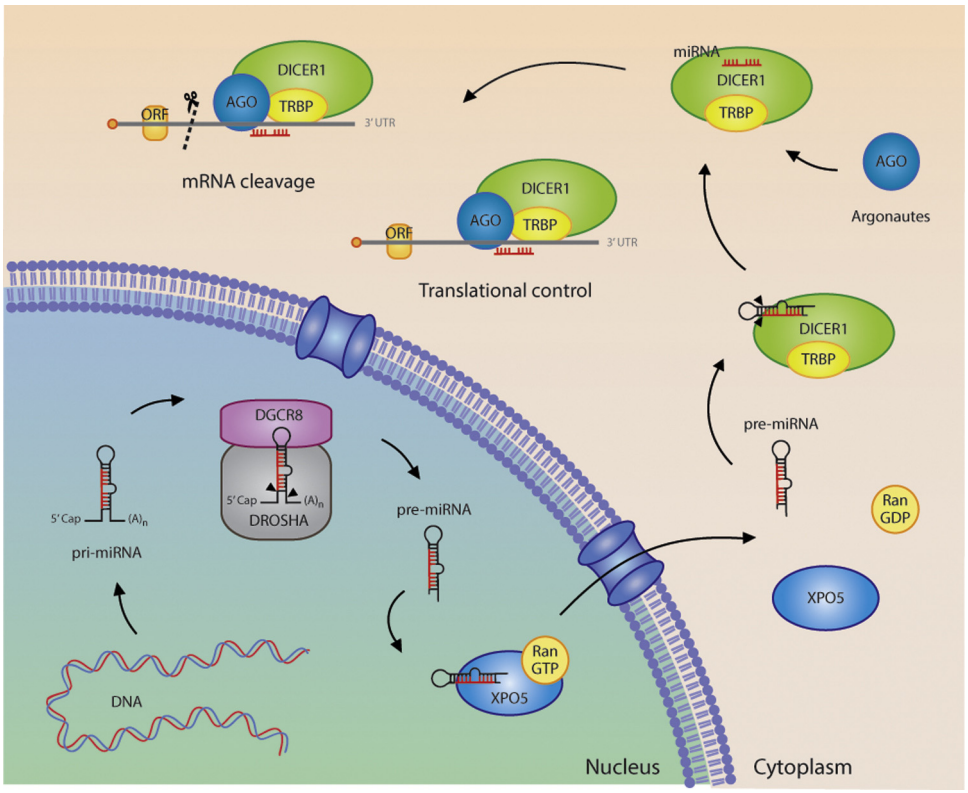

Fig. 1. MicroRNA biogenesis – Canonical miRNAs are transcribed in the nucleus by RNA polymerase II (RNAP II) into primary miRNA (pri-miRNAs) transcripts. These can be single pri-miRNAs or polycistronic pri-miRNAs. The nuclear microprocessor complex composed by Drosha and DGCR8 further process the pri-miRNA into a 72–80 nt precursor miRNA (pre-miRNA). Precursor miRNAs are then exported to the cytoplasm by exportin 5 (XPO5)/CRM1 and are processed into mature miRNAs by Dicer1 and TRBP. One of the strands is preferentially incorporated into the RNA Induced Silencing Complex (RISC) composed of Dicer1/TRBP/AGO2 (argonaute 2). The classical function of the mature miRNA incorporated into the RISC complex is to mediate post-transcriptional inhibition by 3′UTR targeting; nonetheless mRNA cleavage is also possible.

the RNA-induced silencing complex (RISC) [3]. The expression of the target mRNAs is silenced by miRNAs on the RISC complex, either by mRNA cleavage or by translational repression [3].

In mammals, Argonaute 2 (AGO2), which has robust RNaseH-like endonuclease activity, can support Dicer processing by cleaving the 3′ arm of some pre-miRNAs, thus forming an additional processing intermediate called AGO2-cleaved precursor miRNA (ac-pre-miRNA) [8]. Processing of pre-miR-451 also requires cleavage by AGO2, but is independent of Dicer and the 3′ end is generated by exonucleolytic trimming [9].

2. Nuclear export of precursor microRNAs

Transport of RNA through nuclear pore complexes (NPCs) requires a family of conserved nuclear transport receptors (also known as karyopherins), which recognize a short peptide signal on a cargo protein – either a nuclear localization signal (NLS) or a nuclear export signal [10,11]. Typically, karyopherins that import cargo are called importins and karyopherins that export cargo are called exportins. As mentioned before, biogenesis of miRNAs is divided into the nuclear and cytoplasmic phases, and, thus, the nuclear export of their pre-miRNAs is mandatory for maturation. Pre-miRNAs are transported from the nucleus to the cytoplasm by exportin-5 (XPO5), a member of the karyopherin family of proteins (Fig. 1) [12,13].

A feature of karyopherins, and therefore of XPO5, is its regulation by the small GTPase Ran [14]. Ran exists in a GTP-bound state in the nucleus and a GDP-bound state in the cytoplasm. The RanGTP-RanGDP gradient across the nuclear membrane is generated by the action of two regulators, RanGEF (Ran-GDP-exchange factor) in the nucleus and RanGAP (Ran-GTPase-activating protein) in the cytoplasm, and creates a driving force for directional nucleocytoplasmic transport processes (Fig. 2) [15]. Exportin 5 binds nuclear pre-miRNAs only together with RanGTP, and this ternary complex is translocated to the cytoplasm, where it dissociates upon hydrolysis of RanGTP by RanGAP. Upon binding to RanGTP through the amino- and carboxyl-terminal regions, XPO5 holds the double-stranded stem region of the pre-miRNA through an interaction based on different surface charges on XPO5 C-terminal region. Therefore, XPO5 recognizes the structural features shared by different pre-miRNAs as signals for nuclear export, which are formed during the nuclear processing of pri-miRNAs by Drosha and DGCR8 [16].

Biogenesis and nuclear export of miRNAs are coupled at several levels [17]. The key enzyme involved in this coupling is Drosha, which generates a double-stranded RNA minihelix with a ~2-nucleotide 3′ overhang, the unique structure of which is recognized both by XPO5 and the downstream-acting processing enzyme Dicer. Thus, a strict linkage of all processing and export steps ensures the high specificity of miRNA production and function.

2.1. Impairment of precursor miRNA export in cancer

Cancer cells often express substantial amounts of mRNA isoforms with shorter 3′ untranslated regions (3′UTRs) feeding the concept that cancer cells avoid regulation of their transcriptome by miRNAs [18]. States of increased proliferation or cellular transformation are associated with widespread occurrence of the production of mRNAs with shortened 3′UTR and fewer miRNA target sites, indicating that a global switch of the use of