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Review

Circulating miRNAs from blood, plasma or serum as promising clinical biomarkers in oral squamous cell carcinoma: A systematic review of current findings

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ABSTRACT

The purpose of this systematic review was to summarize current findings on the use of circulating miRNAs from blood, serum and plasma as cancer biomarkers in patients with oral squamous cell carcinoma. Studies were gathered after searching four different electronic databases: PUBMED, SCOPUS, Cochrane Library and Web of Science. Additional search was carried out through cross check on bibliography of selected articles. After the selection process made by two of the authors, 16 articles met the inclusion criteria and were included in the review. Results showed that circulating miRNAs from blood, serum or plasma represent promising candidates as cancer biomarkers in patients suffering from oral cancer. The possibility to predict recurrences and metastases through follow-up quantification of candidate miRNAs represents another potential feature to be addressed in future studies. However, methodological standardization and uniform sampling is needed to increase the power and accuracy of results.

Introduction

Background

Oral squamous cell carcinoma (OSCC) is the most common cancer type arising in the oral cavity and belongs to the more wide group of head and neck cancers (HNCs), the sixth most common cancer type in humans [1]. An incidence of 300,000 OSCC cases worldwide has been estimated, accounting for about 24% of all HNCs [2]. The five-year survival for OSCC patients remains less than 50 percent, and late diagnosis may be responsible for the poor prognosis in these patients [3,4]. In fact the five-year survival rate for patients with stage I disease is about 80%, but strongly decreases to approximately 40% in higher stages [5]. No reliable biomarkers are, however, available to detect OSCC aggressiveness and predict disease outcome. In the future new diagnostic and prognostic systems must be developed, in order to allow personalized therapies for individual tumors [6].

microRNAs (miRNAs) are a class of small non-coding RNAs 19–23 nt long, able to negatively regulate target messenger RNAs (mRNAs) by partially binding to their 3′ untranslated region [7]. Until today, about 2500 miRNAs have been recognized, targeting about two thirds of all human genes [8,9]. miRNAs seem to be generated from intergenic genomic sequences or intronic regions of protein-coding genes [10]. The majority of mammalian miRNAs are transcribed by the RNA polymerase II into a long precursor containing a typical stem-loop structure (pri-miRNAs) (Fig. 1) [11]. These initial transcripts undergo cleavage by the ribonuclease enzyme III Drosha to produce the pre-miRNAs comprising around 70 nucleotides and a hairpin structure [12]. Pre-miRNAs are then transported into the cytoplasm by a nuclear export factor (Exportin-5), where they are cleaved by an enzyme called Dicer into duplexes of 21–23 nucleotides [13]. Afterwards, one strand of the duplex, the passenger strand (miRNA*), is degraded and the other incorporated together with Argonaute proteins or lipoproteins to form the RNA-induced silencing complexes (RISC) leading to the formation of mature miRNA [14]. It has, however, been reported that in some cases the miRNA* strands escape the degradation and are loaded on carrier proteins and act as mature

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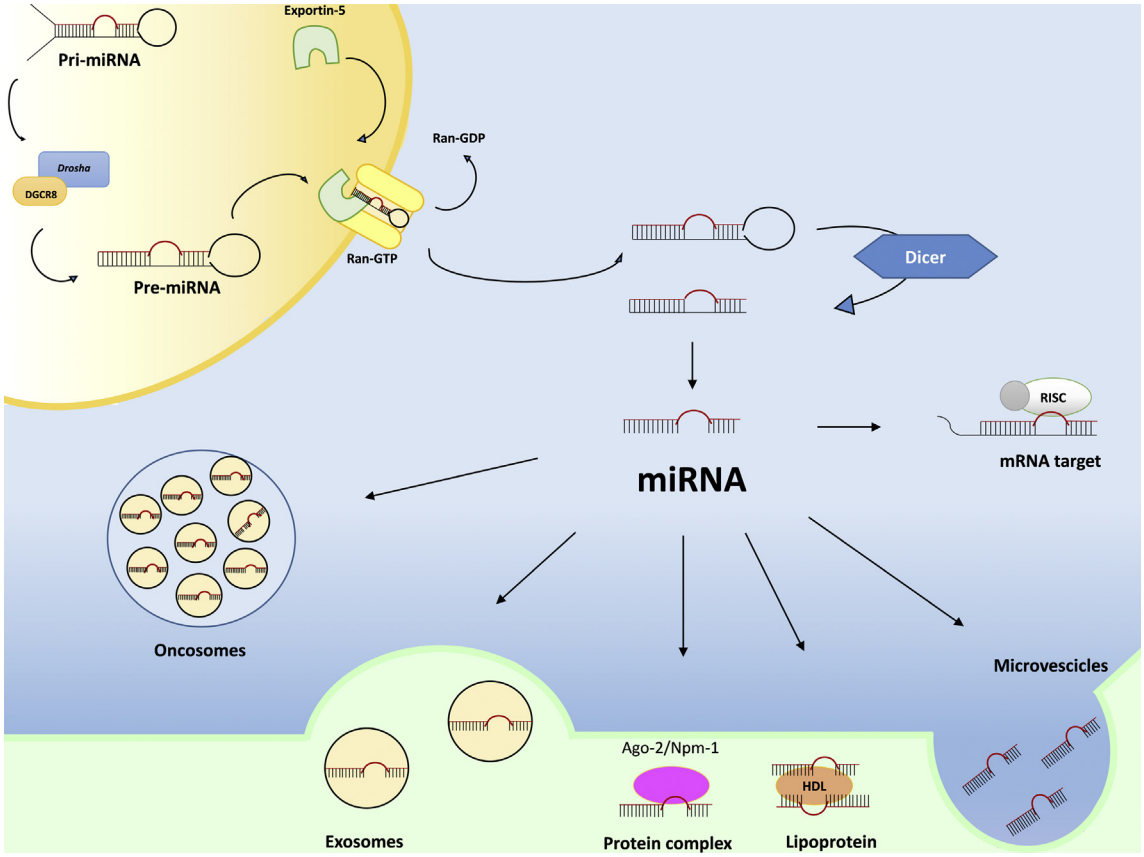

Fig. 1. Scheme of processes involved in synthesis of miRNAs. The process starts in the nucleus and ends with secretion once the miRNa has reached maturity.

miRNAs [15]. Due to their short structure, a single miRNA is able to bind to a lot of target genes and regulate their expression. But a single 3′ untranslated region can also be targeted by a great number of different miRNAs [16]. In cancer miRNAs are often dysregulated and implicated in different cellular process such as differentiation, proliferation, apoptosis and metastasis [17–20]. The presence of miRNAs was also discovered in blood and several other body fluids including: urine, tears, pleural effusions and saliva, exhibiting different expression patterns in cancer patients compared to healthy individuals [21,22]. The exact mechanism with which miRNAs are released into the body fluids is, however, still unclear but various mechanisms seem to be involved [23]. Passive release into body fluids as a result of cell death is supported by the fact that extracellular miRNA levels in conditioned media increased with higher rate of cell death [24]. This does, however, not seem to be the only way for miRNA release as they also can be loaded into microvesicles (also called oncosomes) through membrane budding, or incorporated in exosomes and then released in the extracellular space [25–28] (Fig. 1).

Exosomes are microvesicles of 30–120 nm, initially considered cell waste products, but more recently having gained a lot of interest as they seem to play an important role in intercellular communication [29,30]. Exosomes can be released into the extracellular environment through inward budding of the cell membrane into the cytoplasm, or invagination into multivesicular bodies as intraluminal vesicles (ILVs) and then released after the fusion of ILVs with the cell membrane [31–33]. Exosome-miRNAs have been reported to represent a subset of about the 3% of the entire amount of cell-free miRNAs [24]. A large amount of miRNAs are released through an RNA-binding protein dependent pathway thanks to the association of miRNAs with Argonaute-2 (Ago-2), high density lipoprotein (HDL) and nucleophosmin 1 (Npm1) [23,34–36]. Due to the exosome-carriage and binding to RNA-binding proteins miRNAs have demonstrated to be highly stable in body fluids leading to rising hopes for their future use as cancer biomarkers [37]. In fact it has been seen that circulating miRNAs are not digested by RNase and stable at high pH, boiling and multiple freeze-thaw cycles [27,34,38].

Challenges in the research of circulating-miRNAs

Despite the stable features of miRNAs and the encouraging initial results, researchers were faced with different challenges for the routine use of circulating miRNAs as cancer biomarkers. In fact, before the routine utilization of circulating miRNAs for clinical purposes, a number of issues must be solved for better standardization and safer use in the clinical practice. Firstly inconsistent levels of miRNAs have been reported among different investigated samples [39]. For example serum has been reported to show higher miRNA concentration compared to corresponding plasma, suggesting that the coagulation process may affect the total amount of miRNA [40]. Other issues related to differences in processing and handling of the sample may also lead to significant variation in results [41]. The variation in speed and duration of centrifugation may influence number of platelets and microvesicles remaining in the supernatant, and platelets may in turn unleash their amount of miRNA leading to a variation in total amount of cell free-miRNAs [42]. In addition, when studies are carried out on blood/plasma/serum samples each procedure should aim at reducing the risk of hemolysis as rupture of erythrocytes strongly can increase the amount of miRNA [37,38]. To reduce this risk, the time from blood collection to centrifugation should not exceed 2 h [43]. A