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Review

MicroRNA and esophageal carcinoma

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Abstract

Objective: An abundant class of non-coding small RNA molecules, 21-25 nucleotide in length, are widely found in animals and plants and named microRNA(miRNA)[1-2]. MiRNAs are highly evolutionarily conserved, expressing in specific tissue and timing[2], and negatively regulate the gene expressions at the posttranscriptional level[3], and subsequently control crucial physiological processes such as metabolism, amplification, differentiation, development and apoptosis[4-7]. Therefore, miRNAs could provide an access to many human diseases in theory. Recent evidence demonstrates that miRNAs play an important role in the initiation and progression of human cancer, mainly by interrupting the cell cycle at the cellular level and by interacting with signaling [7-11] The expression profiling of miRNAs can be used as a tool of diagnosis, staging, prognosis and biotherapy of some tumors, as has already been proven to have superiority to mRNA, in the categorization of tumors. This review focuses on the genesis, mechanism of action of miRNA and its relationship to tumors, detection methods and its potential effect on the diagnosis, staging, and biotherapy in esophageal carcinoma.

Keywords: microRNA(miRNA); tumor; oncogenes/tumor suppressor genes; esophageal carcinoma.

INTRODUCTION

MicroRNAs(miRNAs) are small noncoding RNAs of 21-25 nucleotides, the human genome encodes hundreds of miRNAs [12], which posttranscriptionally repress the expression of target genes which encode mRNAs. Bioimformatic data indicate that miRNAs are involved in almost all basic signaling pathways, including the expression of many important tumor-related genes [13]. The recent studies demonstrate that aberrant miRNA expression is closely associated with tumorigenesis, and miRNAs-expression profiling in some tumors has successfully been identified such as the signatures of diagnosis, staging and response to biotherapy. Esophageal carcinoma is one of the six most common malignant tumors in the world, the mortality of which occupies the fourth position among the malignant tumors in China (17.38per100000). The treatment strategies of esophageal carcinoma remain: the combined therapy of operation, radiotherapy and chemotherapy. In this postgenomic project, although being successful in identifying the virulence genes of esophageal carcinoma at the molecular level [14-16], it is still a tough problem to find the best method to effectively carry out gene therapy.

Features of miRNA

As novel kind of gene expression regulators, in comparison with varieties of other small RNAs and protein enzymes, miRNA has unique characteristics of its generation, mechanism and action.

Most humans code miRNAs in the introns between the encoding genes sequence, while others in the sites between transcripts [12]. The biosynthesis process

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of miRNAs is complex, involving five important procedures and five key protein enzymes, RNA transcriptase Ⅱ (Pol Ⅱ)completes the transcription of miRNA[17]; two nucleinase Ⅲ-(Drasha[18-19]and Dicer[20]) alternatively in the cell nucleus and cytoplasm, fulfill the transformation and modification of miRNA. Drasha needs the cofactor-Pasha (that is DGCR)to achieve the goal; the protein Exportin-5 (dependent upon Ran-GTP) transports pre-miRNA (the miRNA precursor of 70nt) from nucleus to cytoplasm[21-22]. The members of the Argonaute protein family which stay in the dsRNA-induced silencing complex (RISC)[20,23], play a significant role in miRNA's combination with RISC. The mutations(or translocations or deletions) of miRNA genes, and abnormities in miRNA-processing including genes and protein enzymes, can lead to the change of its expression level.

After the miRNA duplex structure entering RISC, one chain becomes mature and functions in the RISC, while the other one is degraded. MiRNA identifies the target mRNA by its 5'tip and then coheres to it, the mismatch between the 5'tip and its targets leads to the regulating mRNA's to the function of miRNA's loss [24]. RISC either cleaves or blocks translation of the target mRNA, depending on the degree of sequence complementarily. Through perfect or almost perfect complementary with target messenger RNAs (mRNAs), miRNAs induce the degradation and inhibit the translation of miRNAs, which resemble the process of siRNA-mediated gene silencing (RNAi)[24-25], and the binding site is in the open read frames of mRNA. This mechanism often happens in plants. Another way is realized through imperfect pairing with target mRNAs which is often adopted in animals. In this path, miRNA binds to 3' -UTR regions [26], this doesn't lead to mRNAs' degradation but posttranscriptionally down-regulates the synthesis of functional protein. Recent studies however, show that miRNAs can also cause degradation of mRNAs by directing their rapid deadenylation, which is irreversible. There are other mechanisms of action awaiting further research.

miRNA and tumors

Cancer is viewed as a complex genetic disease. There is excessive expression of oncogenes and/or the low expression of tumor suppressor genes in the initiation and progression of almost tumors. The studies indicate[28] that nearly half miRNAs are located in the fragile sites and genomic regions of tumor-associated-genes, and post-transcriptionally regulate gene expression which function as oncogenes, or tumor suppressor genes.

miRNAs function as tumor suppressor genes

Calin *et al*[29] for the first time reported the correction between miRNAs and cancer, in which found about 65% of chronic lymphatic leukemia(CLL) patients, 50% of lymphoma mantle cell patients, 16-40% of myelomatosis invalids and 60% of prostatic carcinoma patients were miR-15a and miR-16-1 absent. Later, miR-15a and miR-16-1 were found to negatively regulate the expression of Bcl2[30] which is an anti-apoptosis gene that participates in the pathogenesis of a variety of tumors. The loss of miR-15a and miR-16-1 induces the overexpression of Bcl2, by which the damaged cells can not go to apoptosis and facilitate the formation of tumors.

The MicroRNA let-7 family has been shown to be a typical tumor suppressor. The Ras oncogene found overexpressed in pathogenesis of various tumors such as lung caner and colon cancer(where let-7 was lowly expressed) indicated Ras to be the target of the let-7 miRNAs family [31-32]. Moreover, the reduced expression of the let-7 miRNAs family in human nonsmall cell lung carcinoma(NSCLC) is implicated in bad prognosis, and a shortened postoperation survival rate[10]. Another oncogene-High Mobility Group A2(HMGA2) has been shown to be the target of let-7. HMGA2 proteins are expressed predominantly during embryogenesis, their expression being absent or greatly reduced in adult tissues[33]. HMGA2 has been implicated mainly in the tumorgenesis of benign and malignant mesenchymal tumors, such as hysterocarcinoma and breast cancer [34-35]. Further studies show that the effect of let-7 on HMGA2 is dependent on multiple target sites in the 3' untrans lated region (UTR), and the growth-suppressive effect of let-7 on lung cancer cells was rescued by overexpression of the HMGA2 ORF without a 3'UTR. Likewise, chromosomal translocations previously associated with human tumors disrupt the repression of High Mobility Group A2 (HMGA2) and by let-7 miRNA which promotes anchorage-independent growth-a characteristic of oncogenic transformation [36-37]. Interestingly, a study of head and neck squamous cell carcinoma (HNSCC)[37] demonstrated that HMGA2 expression is associated with enhanced selective chemosensitivity towards the topoisomerase (topo) II inhibitor, doxorubicin, in cancer cells. Also that HMGA2 expression is regulated in part by miRNA-98(miR-98) which functions as an oncogene, which also supports the theory that one mRNA may be regulated by

many miRNAs.

Micheal *et al* [38] detected that the expression of miR-143 and miR-145 were obviously downregulated in colon carcinoma tissues as compared with responding normal tissues. Other studies found the same phenomenon in breast cancers and prostatic carcinoma.

Evidence indicates that estrogen powerfully prompts caryokinesis and overexpresses in the tumor tissue of female reproductive system. The carcinogenesis of estrogen has been found to be mediated by estrogen receptor alpha (ERα)in animal experiments, and ERαoverexpression has been identified in most breast cancer. A further study showed two putative miR-206 sites, (hERalpha1 and hERalpha2), which were found in silico within the 3'un translated region (3'UTR) of human ERalpha mRNA. There was an overexpression of pre-miR-206 and reduced ERalpha[39].

miRNAs act as oncogenes

In B-cell lymphoma, cryptic-type lymphoma and mantle cell lymphoma tissues, there is often the amplification of the 13q13 site including c13orf25 genes which encode the miR-17-92 cluster that function as oncogenes[40]. Further studies[41] indicate that the miR-17-92 cluster accelerated c-MYC induced lymphomagenesis, the tumors with an overexpression of c-MYC along with miR-17-92 cluster in comparison with those with only c-MYC overexpression, and has more vigorous progression and a lower death rate. With almost the same effect (as miR-17-92 cluster on c-MYC) miR-155 was found having a joint action with c-MYC overexpressed in B-cell tumors and pancreatic carcinoma[42-44].

Volinia *et al*[45], using a microarray platform, analyzed the microRNAs expression level of six kinds of solid tumors; lung cancer, breast cancer, colon cancer, gastric carcinoma, prostatic carcinoma and pancreatic carcinoma. MiR-21 was found obviously overexpressed in six types of cancer cell, which hint at the general carcinogenesis of miR-21. A recent study showed that miR-21 functioned as an anti-apoptosis and pro-survil factor through directly down-regulating the expression of tumor suppressor gene tropomyosin 1(TPM1)[46].

The study of Voorhoeve *et al* [47]shows the oncogenic function of miR-372 and miR-373 in testicular germ-cell tumors, their mechanism of action is awaiting further research.

The detection methods of miRNA

The detection methods of miRNA appear to change with every passing day since it was first discovered, each strategy having its own strengths and shortcomings. Here we offer an overview, in order to examine these methods.

The primary detection of miRNA was obtained through the isolation and cloning of micromolecular RNA in the cell. Later, Northern blotting hybridization technique was used to detect miRNAs, that was to set up a cDNA Lib of miRNA by RCR, and then to compare them with the BLAST, (the genomic data pool to identified miRNAs profiles) through Northern imprinting [48]. But this method was just adopted for the miRNA genes which were frequently and abundantly expressed in cells. When referring to those miRNAs expressed in specific tissue and timing, bioinformatics technology is applied in order to make a summary of the characters of the miRNAs, then to search for the genomic sequences by computer to identify the expression profiling of the miRNAs. Later, the researchers set their sights on a miRNA precursor, and a novel method was developed by Schmittgen *et al* [49] who reported the expression of miRNA precursor through quantitative real time PCR(RT-PCR). Lee *et al*[50] used RT-PCR to show miR-221, miR-376a and miR-301 significantly overpressed in pancreatic cancer tissues, while Chen *et al*[51] used the same method to monitor the expression of active miRNA.

With the development of microarray technology, the miRNA microarray was initially developed by Liu *et al*[52] which detected miRNA expression profiling through a microarray composed of more than 200 oligonucleotides, and subsequently became the most commonly used high-throughput technique for the assessment of hundreds of cancer-specific miRNAs expression levels in a large number of samples. Calin *et al* [29] for the first time reported the miR-15a and miR-16-1 which function as oncogenes in CLL. Michael *et al* [38] found the notable down-regulation of miR-143 and miR-145 in colon carcinoma with gold-silver microarray. Volinia *et al* [45] showed miR-21 overexprassed in six types of solid tumor cells, and in miR-17-5p and miR-191, which were overexpressed in five.

In 2005, the bead-based miRNA profiling technique, with high specificity was developed by Lu *et al* [11] for systematic analysis of 334 kinds of lymphadenomas and solid tumors, and from here it is believed that distinct miRNA profiles characterize the state of cellular differentiation.

Vinther *et al* [53] first used stable isotope labeling by amino acids in cell culture (SILAC)to study the

effect of miRNA-1 on the HeLa cell proteome. The expression of 12 of 504 investigated proteins was repressed by miRNA-1 transfection, the function of which was subsequently validated on the protein level.

The potential function of miRNA in the diagnosis, staging and biotherapy of esophageal carcinoma

The cancerization of esophagus was caused by synthetic action of multi-modulation and the accumulation or superposition of polygene alteration. Studies [12-14] demonstrated that the initiation of this tumor was induced by the inactivation of some tumor suppressor genes like Rb and P53 as well as the activation of some oncogenes like H-ras, c-MYC and Hsl-1, the mutation of which is facilitated by the environment, and recently a novel oncogene-GAEC1 has been found in the development of esophageal squamous carcinoma[54]. MiRNA, a novel kind of gene regulator (with high potency) is believed of great importance in the initiation and development of varieties of tumors. Overall, miRNA must have a strong effect in the esophageal carcinoma, unfortunately of which, no data is available at this time. Therefore, this study compares tumor tissue with the adjacent normal tissue transversally. This through the gene microarray technique, to find the distinct miRNA profiles as well as to comparing tumor tissues at different stages to find the distinction of miRNA expression profiles (between stages). In order to complete the diagnosis and staging of esophageal cancer we quantitatively analyzed the expression level of specific miRNAs. Finding that they function as oncogenes if expressing, while if there is low or no expression, we subsequently carried out the described biotherapy (to esophageal carcinoma) on the molecular level through RNAi[25,55], antisense technologies [56] and so on to restore the aberrant miRNAs.

The greatest problem existing currently is the identification of the target genes or proteins of miRNAs, which function as oncogenes and tumor suppressor genes. MiRNA may be involved in several signal pathways to regulate various mRNAs, while several miRNAs accommodate a common mRNA by distinct mechanisms, which cause the further difficulty to the studies. Conversely this is good for the gene therapy because the therapeutic tool is expected to have high specificity but function widely. So another goal of our studies is to identify the target genes of miRNAs in esophageal carcinoma and its regulating mechanism. Once this is solved, researchers should be able to guide the corresponding therapy, and we hope will provide novel and highly convenient and specific methods for tumor screening

The failure of therapy mainly owes to the metastasis of tumor, the role of miRNAs of which is little known. It seems that when miRNAs are found, metastasis can lead to a more serious conclusion in esophageal carcinoma, when it comes to the final diagnosis. Put simply it misses the therapeutic chance. A specific gene has been reported to be involved in the metastasis in esophageal cancer[57]. This study focussed on the role of miRNAs in the metastatic process and how they interact, as well as whether the miRNAs could be a possible direction for therapy.

Another problem is how to carry out gene therapy, and extrapolate it from testing laboratory to clinical application. Due to the complexity and diversity of cancer cells, it is difficult to spread the therapeutic effect but limit it to certain tumors [59]. The main therapeutic method is RNAi which is a powerful tool for gene silencing. The ectogenic and endogenous double-strand RNA (dsRNA) is introduced to cells(and cut into siRNA by RNase Ⅲ Dicer method), then siRNA, endonuclease, exonuclease, unwindase and so on compose RISC which induces the degradation of dsRNA, homologous mRNA, and subsequently represses the expression of target genes. Aberrant miRNA is induced to silence if the miRNA function as an oncogene in the tumor tissue. On the contrary, if we interfere with the expression of oncogene through RNAi if miRNA act as tumor suppressing genes, we will attain the goal of treatment. Other possible studies are drug treatment, the study of nonsmall-cell lung cancer cells and the stem cells in mouse embryos. We found the Dicer expression level dramatically low and down-regulated the miRNAs, acting as oncogenes [59,60] which could be the therapeutic point to be targeted by artificial Dicer analogue. We found also antisensenucleic acid technology can effectively inactivate the pathogenic target miRNA. This strongly suggests established miRNA expression signature could be a potent tool to diagnose and treat human cancers in the future.

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