Interactions between miRNAs and mRNAs of apoptosis genes in lung cancer

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Malignant tumours are characterized by increased cell proliferation compared to control tissues [1]. Cell proliferation mainly depends on the rate of the cell cycle and apoptosis. The expression of certain genes involved in apoptosis depends on miRNAs [2-4]. It is therefore important to establish the degree of influence of miRNAs on the expression of apoptotic genes. Understanding the targets of miRNAs will contribute to the development of diagnostic methods as well as their therapeutic use.

Materials and Methods. Human mRNAs were obtained from GenBank (http://www.ncbi.nlm.nih.gov). The nucleotide sequences of human mature miRNAs were downloaded from the miRBase database (http://mirbase.org). Target genes for miRNAs were determined using the MirTarget program [5]. This program defines the following features of binding sites: the start position of an miRNA binding site with respect to the mRNA sequence, the localisation of miRNA binding sites in the 5′UTRs, CDSs and 3'UTRs of genes, the free energy of hybridisation (ΔG, kJ/mole), and the schemes of nucleotide interactions between miRNAs and mRNAs. The ΔG/ΔGm (%) ratio was estimated for each binding site, where ΔGm is equal to the free energy value of miRNA binding to its perfect complementary nucleotide sequence. The miRNA binding sites identified had ΔG/ΔGm of more than 90%.

Results and Discussion. We have established that miRNAs bind with high efficiency to mRNAs of apoptosis genes (table 1). The *CASP10* mRNA has the largest number of miRNA binding sites. miR-1273g-3p, miR-619-5p, miR-1273h-3p, miR-5585-5p are, however, unique among the ten miRNAs identified for the *CASP10* mRNA, in that it affects the expression of over 300 genes with $\Delta G/\Delta G_m$ ratios of 90% or more [6, 7]. miR-1273h-3p has two binding sites on the *CASP10* mRNA and one of them is complementary. Therefore, *CASP10* gene expression is strongly regulated by miR-1273h-3p and other miRNAs. Nine miRNAs have binding sites on the *DFFA* mRNA, four of which are unique: miR-5096, miR-619-5p, miR-1273g-3p and miR-5095. The miR-5096 miRNA also controls *FLIP* and *APAF1* gene expression, and their mRNAs have eight and seven miR-5096 binding sites,

respectively. The *NFKB1* mRNA is a target for six miRNAs. The expression of each mRNA of the *IKK*, *p53*, and *DFF40* genes is regulated by five miRNAs.

Table 1. Characteristics of miRNA binding sites in mRNAs of apoptosis genes

Gene	Characteristics of miRNA binding sites
AKT1	miR-1227-5p, 154◦, 92; miR-1275, 2020•, 93; miR-3620-5p, 164◦, 94; miR-4292, 172◦, 90; miR-4486, 159◦, 91; miR-4507, 161◦, 91; miR-4690-5p, 156◦, 90.
APAF1	miR-1273g-3p, 4931•, 91; 5230•, 96; miR-1972, 5169•, 93; miR-4532, 124◦, 94; miR-520d-5p, 1527▪, 90; miR-5585-5p, 5012•, 91; miR-619-5p, 6736•, 95; miR-6873-3p, 552◦, 91.
BCL2	miR-1343-5p, 606▪, 90.
BCL2L1	miR-3654, 2107•, 90; miR-6842-5p, 1692•, 93; miR-877-5p, 148◦, 92.
BID	miR-133a-3p, 810▪, 91.
CASP10	miR-1260a, 2595•, 91; miR-1260b, 2594•, 92; miR-1273g-3p, 2588•, 93; miR-1273h-3p, 2233•, 91; 2622•, 100; miR-1285-3p, 2356•, 91; miR-4463, 5821•, 91; miR-4516, 5822•, 91; miR-5585-3p, 3388•, 93; miR-619-5p, 3246•, 93; miR-7851-3p, 2656•, 91.
CASP3	miR-4265, 2379•, 90.
CASP7	miR-4507, 167▪, 91.
CASP9	miR-4747-3p, 1068▪, 92; miR-4762-5p, 410◦, 90.
CFLAR	miR-1273g-3p, 3666•, 96; miR-1285-3p, 5665•, 91; 6569•, 91; miR-4258, 4960•, 92; miR-4430, 5006•, 92; miR-5095, 6324•, 91; miR-5096, 2426•, 92; 5125•, 91; miR-5684, 3660•, 90; miR-619-5p, 2352•, 95; 6330•, 95.
DFFA	miR-1285-3p, 2038•, 91; 2984•, 96; miR-1303, 2048•, 91; 2994•, 91; miR-4430, 1720•, 92; 2968•, 92; miR-4784, 656▪, 90; miR-5095, 1788•, 98; 2738•, 95; miR-5096, 1594•, 98; miR-5585-3p, 1939•, 98; 3264•, 95; miR-6125, 40◦, 95; miR-619-5p, 1794•, 98; 2744•, 98; 3124•, 95; miR-1273f, 2242•, 92; miR-1273g-3p, 1565•, 93; 2209•, 96; miR-1285-3p, 2192•, 91; miR-1972, 1763•, 93; miR-5684, 2203•, 92.
FADD	miR-1908-3p, 543▪, 90; miR-4258, 542▪, 90.
FASLG	miR-3960, 323▪, 90; miR-466, 1603•, 91;1605•, 91; 1607•, 91; 1611•, 91; 1613•, 93.
IKBIP	miR-3155b, 154▪, 92; miR-4430, 2307•, 92; miR-5095, 2077•, 91; miR-5585-3p, 2224•, 96; miR-619-5p, 2083•, 91.
IRAK1	miR-1273h-3p, 2895•, 93; miR-5095, 2694•, 95; miR-619-5p, 2700•, 98.
MAP3K14	miR-1227-5p, 1042▪, 92; miR-3713, 836•, 90; miR-4497, 3029•, 90.
NFKB1	miR-1227-5p, 227◦, 94; miR-1825, 1647•, 92; miR-3178, 305◦, 90; miR-4516, 161◦, 91; miR-4685-3p, 42◦, 93; miR-4758-5p, 305◦, 94.
TNFRSF1A	miR-4271, 51◦, 92.
TP53	miR-1227-5p, 648•, 92; miR-1273c, 2296•, 91; miR-1273g-3p, 2316•, 91; miR-1273h-3p, 2350•, 91; miR-1285-3p, 2300•, 95.
TRADD	miR-3155b, 918▪, 90.
TRAF2	miR-4270, 75▪, 91; miR-4418, 953▪, 91; miR-5708, 1595•, 90.

Notes: The first number after miRNA is the binding site position in mRNA (nucleotides); the second number is the ratio ΔG/ΔGm (%); the symbols "▪", "•", "◦" indicate to of binding sites in CDS, 3'UTR and 5'UTR.

In general, the expression of key apoptotic genes is under the control of many miRNAs, which indicates their important role in the regulation of this process. Translational inhibition of these genes leads to a decrease in apoptosis, and decreasing miRNA concentration leads to

increased apoptosis. Therefore, changes in the expression of these miRNAs and consequently, their target genes, will promote oncogenesis. The *TP53* target gene plays a role in the cell cycle as well as in apoptosis. In addition, there are miRNAs that inhibit the translation of *TP53* mRNA, and other common miRNAs (88 miRNAs identified) are also specific for the regulation of apoptosis and the cell cycle. There are several such families of miRNAs: miR-1260, miR-1268, miR-1273 and miR-6511. The list also includes the aforementioned unique miRNAs: miR-5096, miR-619-5p, miR-1273g-3p and miR-5095 [6, 7]. The extent of miRNA regulation of apoptotic and cell cycle gene expression varies depending on the miRNA. This is evident in the observation that the majority of miRNA binding sites are found on target mRNAs of cell cycle genes rather than apoptotic genes. For example, miR-1273f has binding sites only in the mRNA of the apoptotic gene *DFFB* and five cell cycle genes (*E2F2, MDM2, SMAD2*, *SMAD4* and *TFDP2*). Therefore, in this case, miRNA overexpression would have a greater effect on suppressing the cell cycle. The number of target sites in cell cycle or apoptosis-related genes could primarily determine their effect on these processes. It was shown in some cases that miRNA concentration correlates with target gene expression. For example, miR-663 is highly expressed in lung cancer, and is involved in the cell cycle via direct or indirect regulation of *TGFB1, P53*, *BAX* and *FAS* genes. It was speculated that miR-663 plays an important role in the development of lung cancer and can be used in its treatment as a regulator of target gene expression [8]. Overexpression of miR-26a in A549 human lung cancer cells decreases cell proliferation by blocking the transition between the G1/S phases. This results in induced apoptosis and inhibition of metastasis, and invasion *in vitro* [9]. The decreased expression of miR-101 is associated with *EZH2* overexpression in non-small cell lung cancer (NSCLC). The transfection of miR-101 decreased the expression of *EZH2* and led to a decrease in cell proliferation via apoptosis induction in NSCLC [10]. Increased miR-451 significantly reduced NSCLC cell proliferation in an *in vitro* colony formation assay, as well as decreased metastasis formation *in vivo* by increasing apoptosis via inactivation of the Akt signaling pathway. The expression of miR-451 also significantly inhibited RAB14 protein synthesis; therefore, the interaction between miR-451 and RAB14 was proposed as a possible treatment strategy for NSCLC patients [11]. The expression of miR-100 closely correlates with the

stages of NSCLC development. Patient survival in low miR-100-expressing patients was significantly reduced compared to patients with high miR-100 expression. Furthermore, miR-100 inhibits G2/M transition and increases apoptosis in NSCLC cells [12]. The expression of miR-21 in plasma correlates with TNM stage and metastasis in patients with NSCLC. miR-196a is overexpressed in NSCLC tumour tissue and cell lines, compared to normal tissue. It was shown that miR-196a expression correlates with cell proliferation, migration, and invasion of NSCLC cells. miR-619-5p, miR-1273g-3p, miR-5096, miR-5095, miR-5585-5p may serve as biomarkers that regulate the expression of several oncogenes, since they have binding sites on the mRNAs of apoptotic genes. It is necessary to control the expression of miRNA target genes for reliable prediction of the miRNAs involved in oncogenesis. Early diagnosis of lung cancer can be achieved by the detection of miRNAs in the blood, since mRNAs of their target genes cannot enter into the bloodstream. The expression of miRNAs and their target genes is critical for cancer detection in biopsy, since their expression increases the probability of correct identification of specific genes involved in tumorigenesis in individual patients. This provides a basis for the use of miRNAs in diagnosis as well as therapeutic techniques.

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