The interaction of miRNAs with mRNAs of the cell cycle genes in lung cancer

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One of the specific features of carcinogenesis is increased cell proliferation caused by changes in the rate of the cell cycle and apoptosis [1, 2]. It is therefore important to examine the influence of miRNAs on these processes. Hundreds of genes and miRNAs involved in the development of malignant neoplasms serve as biomarkers of lung cancer. Identifying the association between miRNAs and their target genes is therefore critical for characterising the features of various tumours and their subtypes. In this study, the interaction of miRNAs with the mRNAs of genes involved in the cell cycle was assessed based on the Kyoto Encyclopaedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/).

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 [3]. 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 ΔG is equal to the free energy value of miRNA binding to its perfect complementary nucleotide sequence. The miRNA binding sites identified had ΔG/ΔGm more than 90%.

Results and Discussion. The miRNA binding sites in mRNAs of human cell cycle genes were identified, and are characterised in Table 1. We hypothesized that the expression of most cell cycle genes could be regulated by various miRNAs. Some mRNAs have binding sites for several miRNAs, which control their expression. For example, the *DP-2* mRNA has eight binding sites. Five miRNAs have binding sites in *GSK3β, TGFβ, h300, SMAD4, h53, ATMATR, PTTG* mRNAs, and four miRNAs have three binding sites in *CDH1, MEN1, CDC6, CDK4, E2F1* mRNAs. Each mRNA of the *SMAD3, RAD21, p57, ESP1, AB1, E2F1, E2F5, E2F4, CHK1* genes has three binding sites.

Table 1. The characteristics of miRNA binding sites in mRNAs of the cell cycle genes

Genes	Characteristics of miRNA binding sites
ABL1	miR-149-3p, 3425▪, 90; miR-3685, 5136•, 91; miR-383-3p, 4007•, 90; miR-4519, 3648▪, 90.
ATM	miR-1273a, 11053•, 90; miR-1273e, 11118•, 93; miR-1273g-3p, 11075•, 96; miR-5585-5p, 11155•, 91; miR-619-5p, 9792•, 98; miR-6507-5p, 2162▪, 90; miR-6829-3p, 266◦, 91.
CDC25A	miR-6749-3p, 1879▪, 90; miR-6809-3p, 1885▪, 94.
CDC25B	miR-4463, 457◦, 96; miR-4487, 756◦, 90; miR-6124, 664◦, 94; 668◦, 90.
CDC6	miR-1273g-3p, 2286•, 93; miR-566, 2376•, 90; miR-5684, 2280•, 92; miR-6833-3p, 102◦, 90.
CDC7	miR-4486, 1281▪, 91; miR-765, 81◦, 91.
CDH1	miR-1273c, 3250•, 91; miR-1273g-3p, 3270•, 93; miR-1273h-5p, 3304•, 96; miR-3656, 486▪, 90; miR-4430, 51◦, 94; miR-7160-3p, 185▪, 91.
CDK4	miR-1285-5p, 1940•, 92; miR-5095, 1694•, 91; miR-5096, 1774•, 96; miR-619-5p, 1700•, 95.
CDKN1C	miR-3714, 561▪, 90; miR-4463, 810▪, 91; 864▪, 94; 870▪, 94; 882▪, 91; 888▪, 91; 900▪, 91; miR-4505, 904▪, 90; miR-762, 739▪, 92; 745▪, 91; 805▪, 94; 811▪, 94; 817▪, 91; 901▪, 92.
CDKN2D	miR-3940-3p, 133◦, 90; miR-4274, 115◦, 92; miR-6769a-3p, 734▪, 91.
CHEK1	miR-4271, 63◦, 90; miR-5585-3p, 2574•, 93; miR-619-5p, 2567•, 95.
E2F1	miR-1913, 29◦, 90; miR-3960, 89◦, 92; miR-4749-3p, 2322•, 91; miR-6511a-3p, 2327•, 91; miR-6511b-3p, 2326•, 93; miR-6786-5p, 268▪, 90; miR-6813-3p, 2537•, 91.
E2F2	miR-1273f, 4160•, 92; miR-1273g-3p, 4127•, 96; miR-4534, 39◦, 96; miR-4539, 1407▪, 90; miR-548m, 2091•, 90; miR-5684, 4121•, 92; miR-760, 625▪, 93.
E2F4	miR-4265, 852▪, 90; miR-6791-3p, 160▪, 91; miR-7704, 80▪, 93.
E2F5	miR-1268a, 143▪, 90; miR-6068, 104▪, 90; miR-6791-3p, 233▪, 91.
ELL	miR-4800-5p, 2827•, 91; miR-6777-3p, 2964•, 93; miR-6817-3p, 2923•, 92.
EP300	miR-1908-3p, 155◦, 90; miR-2682-3p, 298◦, 90; miR-3960, 52◦, 90; miR-574-5p, 8795•, 93; 8801•, 93; 8803•, 93; 8805•, 93; 8807•, 93; 8809•, 93; 8811•, 93; 8813•, 93.
ESPL1	miR-6505-3p, 576▪, 90; miR-6735-3p, 1878▪, 95; miR-6815-3p, 3126▪, 91.
GSK3B	miR-1268a, 361◦, 92; miR-1268b, 359◦, 91; miR-3960, 9◦, 92; 12◦, 92; miR-466, 4712•, 91.
MAD1L1	miR-4489, 2505•, 91; miR-6078, 1943▪, 98; miR-6132, 2467•, 90;
MDM2	miR-1273e, 2520•, 93; miR-1273f, 6771•, 92; miR-1273g-3p, 2116•, 96; 2485•, 91; 6738•, 96; miR-1285-3p, 3217•, 91; miR-3929, 3012•, 93; miR-5684, 2479•, 90; 6732•, 90.
MYC	miR-1227-5p, 28◦, 94; miR-6761-5p, 989▪, 91.
RAD21	miR-1322, 1575▪, 92; miR-3656, 186◦, 90; miR-4762-5p, 320▪, 90; miR-6124, 191◦, 92.
RB1	miR-3960, 224▪, 92; miR-4736, 277▪, 90.
RBL1	miR-5095, 3528•, 93; miR-5096, 3608•, 96; miR-619-5p, 3534•, 93; 3668•, 96.
REEP5	miR-574-5p, 1477•, 93; 1479•, 93; 1485•, 93; 1487•, 93; 1489•, 93; 1491•, 93; 1493•, 93.
SMAD2	miR-1273f, 6124•, 90; miR-566, 6181•, 92.
SMAD3	miR-1227-5p, 4◦, 90; miR-4492, 107◦, 94; miR-4507, 2065•, 91; miR-4508, 110◦, 94; 242◦, 90; miR-4690-5p, 2065•, 92; miR-6089, 2077•, 91.
SMAD4	miR-1273f, 4344•, 92; miR-1273g-3p, 4311•, 95; miR-1972, 4551•, 90; miR-5579-5p, 5307•, 90; miR-574-5p, 7741•, 91; 7743•, 93; 7745•, 93; 7747•, 93; 7749•, 93; 7751•, 93; 7755•, 91.
SMC1A	miR-1282, 2128▪, 90; miR-3119, 3496▪, 90.
TFDP2	miR-1273f, 5323•, 90; 5858•, 92; 7373•, 92; miR-1273g-3p, 5292•, 98; 5824•, 91; 7341•, 96; miR-1285-5p, 9171•, 91; miR-1303, 4500•, 93; miR-5096, 9004•, 96; miR-5585-3p, 4393•, 91; miR-5684, 5286•, 92; 7335•, 92; miR-619-5p, 6779•, 95; 8930•, 96; 9064•, 96.
TGFB1	miR-1234-5p, 2089•, 90; miR-3141, 873◦, 90; miR-4274, 254◦, 90; miR-4508, 2060•, 90; miR-4530, 218◦, 92; miR-4651, 2086•, 95; miR-6089, 2064•, 91; miR-6125, 1◦, 91; miR-6742-5p, 2047▪, 90; miR-6824-5p, 707◦, 90; miR-6877-5p, 4◦, 90; miR-877-3p, 232◦, 93.
TP53	miR-1273c, 2296•, 91; miR-1273g-3p, 2316•, 91; miR-1273h-5p, 2350•, 91.

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 binding sites in CDS, 3'UTR and 5'UTR.

The mRNA of target genes with revealed miRNA binding sites can significantly alter the rate of the cell cycle. Unique miRNAs like miR-619-5p, miR-1273f, miR-1273g-3p, miR-574-5p, miR-3960, miR-619-5p, miR-1273e, miR-5096 and miR-5095 [4, 5] have binding sites in mRNAs of several genes. Consequently, there is a high probability that these miRNAs have important functions in tumourigenesis. It is necessary to note that some miRNAs may function as oncogenes or tumour suppressors. This bidirectional action of miRNAs complicates the unambiguous interpretation of their action; however, changes in their expression may impact the rate of the cell cycle and mediate tumorigenesis. Some of the genes influenced by miRNAs are transcription factors that can alter the expression of oncogenes and tumour suppressors. *ATM, MDM2, CDH1, CDC6, EF2, SMAD1, SMAD4, TFTP2* and *TP53* genes participate in regulation of the cell cycle, and its mRNA is a target for miRNAs of the miR-1273 family. The studied genes are involved in the development of cancer at various locations. For example, *MDM2*, an oncogene-encoded cellular phosphoprotein, negatively regulates p53 by blocking p53-mediated transactivation. This gene plays a significant role in human sarcomas, where the p53 wild-type allele is preserved. Therefore, it was proposed that *MDM2* neutralizes the function of p53 in oncogenesis [6]. The *MDM2* gene is a target for three miRNAs of the miR-1273 family (miR-1273e, miR-1273f, miR-1273g-3p). miR-1273g-3p has multiple binding sites on the *CDH1* mRNA, and *CDH1* gene mutations correlate with tumorigenesis in different tissues, including the development of non-small cell lung cancer. The loss of function of its encoded protein leads to tumour progression via increased proliferation, invasion, and metastasis [7]. The *CDH1* mRNA is a target for three miRNAs of the miR-1273 family (miR-1273c, miR-1273g-3p, miR-1273h-5p), and the *CDH1* gene is specifically related to the development of large-cell lung carcinoma. The majority of miR-3960 binding sites are located in 5′ UTRs and CDSs. The *E2F1* gene is involved in cell cycle regulation, and its mRNA is a target for miR-3960 and miR-574-5p. The E2F protein family plays a key role in cell cycle control and in the function of tumour suppressor proteins [8]. *E2F1* as well as *E2F2, MYC, SMAD4* and *TP53* genes are responsible for the development of small cell lung cancer [9, 10]. The protein encoded by the *RB1* gene inhibits the cell cycle. The EP300 protein acts as a tumour suppressor in lung cancer neoplasm and its mRNA has multiple miR-574-5p binding sites. It

is known that miR-574-5p is highly expressed in lung cancer. miR-619-5p, miR-5096 and miR-5095 have binding sites on the *ATM, CDK4, CHEK1, RBL1* and *TFDP2* genes mRNAs. Increased expression of the *CDK4* gene is an indicator of the aggressiveness of the tumour [11]. *CHEK1* gene expression correlates with poor survival of patients with primary lung adenocarcinomas [12]. These results demonstrate that unique miRNAs of defined concentrations can strongly influence the expression of genes involved in cell cycle regulation. Increased levels of miR-1273f, miR-1273g-3p, miR-1273e, miR-574-5p, miR-3960, miR-619-5p, miR-5096 and miR-5095 serve as an indication of carcinogenesis. Based on the results of the interactions of miRNAs and target genes, we propose that miR-619-5p, miR-1273f, miR-1273g-3p, miR-574-5p, miR-3960, miR-619-5p, miR-1273e, miR-5096 and miR-5095 be used as a marker of carcinogenesis. mRNAs of the *DP-2, GSK3β, TGFβ, h300, SMAD4, h53, ATMATR, PTTG, CDH1, MEN1, CDC6, CDK4, E2F1, SMAD3, RAD21, p57, ESP1, AB1, E2F1, E2F5, E2F4* and *CHK1* genes are targeted by three and more studied miRNAs; therefore, this gene can also serve as a tumour marker since its expression is decreased in the presence of the predicted miRNAs.

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