EJGO European Journal of Gynaecological Oncology

Review Articles

The role of microRNAs in endometrial cancer and influence on future therapy: focusing on miRNA-21

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Summary

MicroRNAs are small noncoding polynucleotides, which are involved in numerous biological processes including cell proliferation, differentiation, embryonic development, as well as regulation of cell death and survival. Recent investigations have shown impact of microRNAs on cancers prognosis and diagnosis. Current review focused on the role of microRNA-21 in cancers tumorigenesis. Endometrial cancer is the most common gynecological malignancy and the fourth most common in general classification of cancers in Western Europe; thus discovering new molecules may become a useful diagnostic tool. Furthermore, in this review, the authors emphasized microRNAs having considerable influence on endometrial cancer development. Finally, they highlighted the role of microRNAs as a target for future therapy and circulating microRNAs as a potential biomarker in malignancies.

Key words: MicroRNA; MicroRNA-21 in cancers; Endometrial cancer.

Introduction

MicroRNAs are small noncoding RNAs, consisting of 19-24 nucleotides, having the ability to regulate expression of the target genes post-transcriptionally. For the first time described in 1993 by Lee *et al.* in *C. elegans* species, over the last two decades have been found in diverse animal genomes. Nowadays it is believed that miRNAs regulate approximately one-third of human genes [1, 2].

MicroRNAs are naturally produced by the cell. Their exact biological functions are still not fully understood, though microRNAs act in major biological processes including cell proliferation and differentiation, embryonic development, as well as in regulation of cell death or survival [3, 4]. Recent research revealed influence of microRNAs on immune system and their involvement in cancer development [5, 6].

MicroRNAs originate from the nucleus where their genes are transcribed by RNA polymerase II yielding primary transcripts called pri-microRNAs. Pri-microRNAs are subsequently cleaved by Drosha RNAse III resulting in formation of pre-microRNAs [7, 8]. The latter ones are transferred from the nucleus to the cytoplasm by exportin 5 [9]. Next, pre-microRNA maturates into double-stranded microRNA followed by strand separation and incorporation of the guide strand into RISC complex (RNA- induced silencing complex). The guide strand of the mature microRNA is crucial for recognition of the target messenger RNA (mRNA), while the remaining strand usually degrades. Mature microRNA directs RISC to cleave the mRNA or repress its translation depending on the degree of sequence complementarity between the "seed sequence" (nucleotides 2-8) of the microRNA and the 3' UTR (untranslated region) of the specific target mRNA. In case of perfect complementarity, microRNA leads RISC to mRNA degradation [10-13].

Significance of microRNA

Interestingly, current findings show that microRNA may act on target mRNA not only by binding its 3' UTR, but also 5' UTR or the open reading frame sequences as well. In contrast to the canonical mechanism of gene silencing through 3'UTR, 5'UTR interaction have been demonstrated to play role rather in activation than suppression of the target genes [14, 15].

Interactions of microRNA seem to be even more complex. Certain ribonucleoproteins may interfere with the "seed sequence" of the microRNA which results in the decrease of binding to microRNA targets. This process, known as decoy activity, is an example of RISC independent microRNA regulatory function [16]. Furthermore, some authors suggest, that microRNA may regulate gene ex-

Revised manuscript accepted for publication September 16, 2015

Table 1. — *Predicted target genes for hsa-miR-21 broadly conserved family according to TargetScane database.*

Target gene	Gene name
TIMP3	TIMP metallopeptidase inhibitor 3
BCL11B	B-cell CLL/ lymphoma 11B
RHOB	Ras homolog gene family, member B
CCR7	Chemokine receptor 7
TIAM1	T-cell lymphoma invasion and metastasis 1
C17orf39	Chromosome 17 open reading frame 39
EIF4EBP2	Eukaryotic translation initiation factor 4E binding protein 2
TAGAP	T-cell activation RhoGTPase activating protein
MAP2K3	Mitogen- activated protein kinase kinase 3
MAP3K1	Mitogen-activated protein kinase kinase kinase 1
TGFBI	Transforming growth factor, beta-induced
EGR3	Early growth response 3
FGF1	Fibroblast growth factor 1
TGFBR2	Transforming growth factor, beta receptor II
PI15	Peptidase inhibitor 15
CDC25A	Cell division cycle 25 homolog A
ABCD2	ATP-binding cassette, subfamily D, member 2
CDK 6	Cycylin- dependent kinase 6
BTK	Bruton agammaglobulinemia tyrosine kinase
TET1	Tet oncogene 1

pression at transcriptional level by direct binding to the DNA or by modulation of the DNA methylation [17, 18].

Each microRNA usually concerns the large number of target genes [19]. To illustrate this, Table 1 presents examples of target genes for microRNA-21 family, according to TargetScan5.2 database [www.targetscane.base.org]. On the other hand, every single gene may be regulated by variety of microRNAs, since the 3'UTR may contain number of potential docking sites. Furthermore, single nucleotide polymorphism in the target gene can alter the binding potential where as polymorphisms in the microRNA genes effect processing of pri-microRNA. Modified cleaving of primary transcript by just one nucleotide may rearrange completely binding site of mature microRNA (named therefore isomicroRNA) and its regulatory impact on targeted genes [20, 21].

MicroRNA-21 and cancers

MicroRNA-21 is known as an oncogene (oncomiR), that is located in the chromosome 17q23 region. One of the first experiments regarding sequence regulated by the microRNA-21 was presented by Gumireddy *et al.* The study conducted on DNA reporter construct, containing sequence complementary to investigated microRNA. They observed altered luciferase signal compared to control with microRNA-21 inhibitor, proving the presence of specific mature microRNA in the transfected cells. Further investigation using quantitative RT-PCR, revealed altered transcription level of microRNA-21 in various cancer cells [22]. Nowadays, undisputed overexpression of the microRNA-21 is found in: colon adenocarcinoma, glioblastomas, breast, prostate, pancreatic, gastric, and lung cancer [23-28].

Clinical studies demonstrated that expression of miRNA in tumor tissue may have prognostic value. For instance, meta-analysis by Pan *et al.* indicated, that increased expression of microRNA-21 is associated with poor survival in patients diagnosed with breast cancer [29]. Another recent research, suggest that microRNA-21 and microRNA-221 are biomarkers determining malignancy of pancreatic cysts. In this study, increased expression of microRNA-21 and microRNA-221 was higher in malignant cysts compared to benign ones. However, only microRNA- 21 was considered to be significantly higher in premalignant cysts ($p = 0.03$) [30]. Likewise, microRNA-21 was considered to be prognostic biomarker for colorectal cancer [31]. Moreover, there are numerous examples of microRNA-21 participation in oncogenesis and other pathological conditions: in renal cancer cell invasion, development of breast cancer, inflammatory bowel diseases or chronic kidney diseases [32-35].

Since there are many potential targets of miRNA-21, identification of the pathogenic pathway, that is chiefly affected by this microRNA is in focus of the ongoing research. Meng *et al.* considered role of phosphatase and tensin (PTEN) homolog pathway and matrix metalloproteases 2 and 9 in pathogenesis of human hepatocellular cancer [36]. Frankel *et al.* reported link between microRNA-21, the p53 tumor suppressor protein, and programmed cell death 4 (PDCD4) in breast cancer [37]. Huang *et al.* highlighted importance of interaction of Krüppel-like factor 9 (KLF9) and microRNA-21 in gliomas progression [38].

The most recent data by Dong *et al*. imply impact of silencing of microRNA-21 in lung cancer cells A549/DDP on inhibition of the cell cycle, reduction of AKT signaling pathway, increased cell apoptosis, and decreased expression of P-glycoprotein associated with multi-drug resistance. These findings may help to select potential targets for future therapy of the patients diagnosed with multi-drug resistant lung cancer [39].

Regarding microRNA-21 and endometrial cancer (EC), overexpression of microRNA-21-3p has been found in EC and correlated with higher expression of L1 cell adhesion molecule, as well as poorer prognosis in this type of the tumor. In contrast to complementary sequence microRNA-21-5p, which increased expression has not been confirmed neither positively correlate with L1 cell adhesion molecule by the authors [40].

EC

EC is the most common gynecological malignancy in Europe and North America, as well as the fourth most common in general classification of cancers in Western Europe. There are two types of EC with different characteristics and prognosis (Figure 1). Type I EC is estrogen-dependent tumor, histologically classified as low-grade and low-stage with a

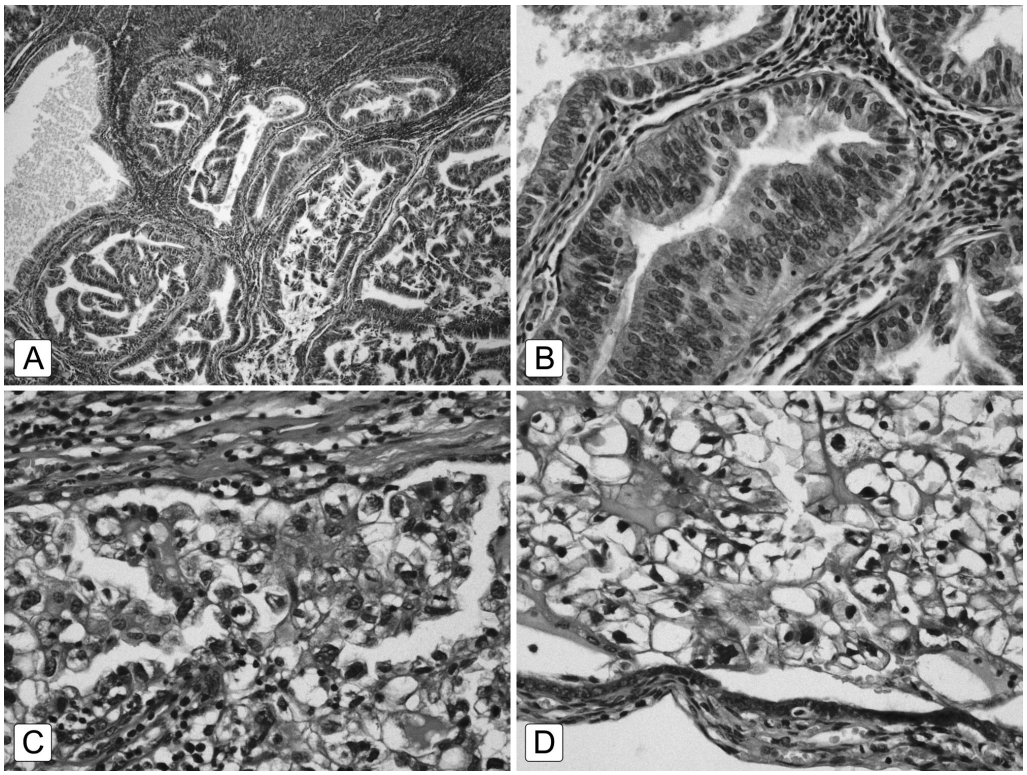

Figure 1. — Morphology of EC type I (A, B) and type II (C, D), H&E staining. A) EC I endometrioid adenocarcinoma, neoplastic glandular cells (tubular in shape) infiltrate myometrium; B) enlargement of glandular tubule consisting of neoplastic cells accompanied by lymphocytic infiltration in the stroma; C) EC II evident neoplastic invasion, increased amount of nuclear atypia with presence of clear cells; D) primary EC with extension to the lumen of fallopian tube.

usually excellent prognosis (Figure 1A, B). In contrast to type I, type II EC is unrelated to estrogen stimulation and is characterized by worse prognosis. Type II EC includes poorly differentiated Grade 3 tumors consisting of serous, papillary or clear cells components (Figure 1C, D). Therefore, the latter one is associated with higher incidence of metastatic disease [41, 42].

MicroRNA in EC

Recent analysis of endometrial cancers by Lee *et al.* suggests dysregulation of over 50 microRNAs. Authors reported mainly the overexpression of microRNA:-141,-182, -200a, -200b, and –205 [43]. Dong *et al.* correlate higher expression of the microRNA-130b, which is a negative regulator of ZEB1, with longer survival of the patients diagnosed with EC. Moreover, this study demonstrated that reduced expression of microRNA-130b triggers ZEB1 axis and leads to cancer cell invasion [44]. Another research published last year, revealed involvement of microRNA-200a, microRNA-205, and miRNA-410 in the disease and suggest them as potential diagnostic and prognostic markers for the rate of recurrence in patients with EC [45].

The study conducted on the group of 73 EC cases reports increased expression of microRNA-141, -200a, -205, as well as decreased expression of microRNA-143 and microRNA-145. Additionally, Wang *et al.* concluded that overexpressed microRNA-141 and microRNA-200a may become a potential target for PTEN pathway in EC tumorigenesis [46]. Furthermore, Lee *et al.* suggest that KRAS-variant allele in the 3' untranslated region rs61764370 (G → T) may become a genetic marker of increased risk for type II EC. As this polymorphism disrupts let-7 binding, which results in KRAS overexpression, can predict the risk of occurrence of the non-small lung cancer or triple negative breast cancer [47].

A novel study by Dong *et al.* show microRNA-106b as a modulator of the epithelial-mesenchymal transition (EMT) and tumor metastasis through TWIST1 in aggressive subtype of the EC. MicroRNA-106b regulate post-transcriptionally TWIST1 mRNA by direct binding to the 3' UTR, which inhibits invasion of the endometrial cancer cells [48]. On the other hand, Konno *et al.* report that microRNA-101 may enhance sensitivity to chemotherapeutics (paclitaxel) in aggressive type of EC. It acts through inverse correlation compared with expression EZH2, MCL-1 and FOS. Increased expression of the microRNA-101 suppress EC cell invasion, proliferation, and induces apoptosis [49].

MicroRNA and future therapy

Due to altered expression of microRNA in cancer cells compared to control healthy tissues, microRNAs seem to play important role in diagnosis and prognosis. MicroRNAs are also potential targets for cancer therapy. Hwang *et al.* demonstrated that during treatment of pancreatic ductal adenocarcinoma cell line with adjuvant therapy, decreased expression of microRNA-21 resulted in reduction of cell growth. Subsequently, transfection of cell line with anti-microRNA-21 im-

proved vulnerability for anticancer drugs [50]. Likewise, previously mentioned data by Dong *et al.* showed improved chemosensitivity after silencing of microRNA-21in lung cancer cell line. At the same time, overexpression of microRNA-21 in colorectal cancer cell lines induced resistance to 5-fluorouracil. Additionally, Valeri *et al.* considered microRNA-21as oncogene acting through downregulation of human mutS homolog 2 (hMSH2) [51].

Detailed understanding of microRNAs role in cancers pathogenesis will enable more accurate and personalized therapy. To overcome microRNAs tissue specific delivery difficulties, several strategies, both viral and non-viral, have been developed: chemical modifications with cholesterol conjugation, peptide nucleic acids or phosphorothioate backbones [52*,* 53]; however none of them shows satisfactory toxic effect *in vivo* [54, 55]. Only nanoparticles and polymers strategies remain promising alternative for antisense delivery to the specific tissues [56]. Recent study by Ekin *et al.* showed efficient transfection of microRNA-145 to prostate and breast cancer cells using gold based nanocarrier [57].

Ultimately, Yoo *et al.* described novel layered gadolinium hydroxychloride (LGdH) nanoparticles as a efficient tool in delivery anti-microRNA-10b to metastatic breast cancer cell line [58]. Nevertheless, further investigations of exact microRNAs delivered by nanoparticles and their influence on carcinogenesis *in vivo* needs to be undertaken. Besides microRNA obtained from solid tissues, circulating microRNAs are promising biomarkers of cancers early diagnosis, tumor progression, and response to treatment [59]. The newest studies have shown presence of tumor specific microRNAs in blood, urine or even in asthma cases - bronchoalveolar lavage fluid. The exact mechanisms of releasing microRNA to extracellular space remains unknown, however scientists consider attendance of particular vesicles- exosomes and microvesicles [60, 61].

MicroRNAs are resistant to plasma RNAses. This property makes circulating microRNAs a perfect candidate for screening tool and diagnosis. Easy approach to fast illness detection is crucial especially for cancer patients: it may indicate earlier treatment implementation and their further survival.

Concerning EC, the latest data indicate circulating microRNA-15b, microRNA-27a, and microRNA-233 as a valuable, non-invasive biomarkers of endometrial endometrioid adenocarcinoma [62]. Dong *et al.* demonstrate microRNA-194 as a novel therapeutic target in invasive EC. The research demonstrates suppressive role of microRNA-194 by direct binding to 3' untranslated region of the oncogene BMI-1, which results in the reduction of the EC cell invasion [63]. Yet, Hiroki *et al.* showed microRNA-34b as a potential tumor suppressor in aggressive type of EC–endometrial serous adenocarcinoma. MicroRNA-34b promotes hypermethylation in targeted genes and its high level of expression reduces invasion of EC cells [64].

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