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MicroRNA-181a Suppresses Progestin-Stimulated Breast Cancer Cell Growth

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ABSTRACT

Despite all our efforts, breast cancer remains a major public health problem threatening women's health all around the world. The morbidity of breast cancer is rising in most countries and is going to rise further over the next 20 years. Hormone treatment is widely used and it is the most efficient method of reducing menopausal symptoms or preventing abortion and pregnancy. However, multiple studies have demonstrated that hormones, especially synthetic progesterone, remain an indisputable risk factor for breast cancer. MicroRNAs are a group of endogenous small non-coding single strand RNAs which play regulatory roles in the initiation, development, and progression of different types of cancer. Evidence from multiple sources indicates that microRNA-181a exerts anti-breast cancer effects by inducing cancer cell death and preventing tumor invasion, infiltration and metastasis, etc. Our recent studies revealed that microRNA-181a not only suppresses breast cancer MCF-7 cell growth but also abrogates progestin-provoked cell growth. In this review, several interesting aspects of hormone replacement therapy and the role of microRNA-181a during progestin treatment are discussed.

Introduction

In recent years, numerous studies have shown that microRNAs (miRNAs) play important roles in tumorigenesis, tumor progression, invasion and metastasis by silencing their target genes. We have also demonstrated that microRNA-181a (miR-181a) suppresses progestin-promoted breast cancer cell growth[1]. In this review, we focus on the interaction between miR-181a and progesterone receptor membrane component 1 (PGRMC1) in breast cancer cells after progestin stimulation. We also elucidated the possible underlying mechanisms of miR-181a which decreased the effect of norethisterone (NET) in breast cancer. The resulting data may provide a novel therapy for breast cancer treatment.

Hormone Therapy Plays an Important Role in Postmenopausal Women

According to the latest 2017 WHO statistics, life expectancy for Canadian women is 84.1 and the average age of menopause is 51, which means that more than a third of women's lifetimes are spent in menopause. Menopause is a natural process but causes many pathological problems. For instance, it can increase the prevalence of coronary heart disease, osteoporosis, mood disorders, and sexual dysfunction[2]. Menopausal Hormone Therapy (MHT) is the most efficient therapy for menopausal symptoms and chronic diseases, but more than 50 studies have found that MHT can increase breast cancer risk[3].

Progestin is Responsible for Increasing Breast Cancer Risk

During MHT treatment, exogenous estrogens can stimulate endometrial cell proliferation, increasing the risk of endometrial cancer. There is a strong link between the use of estrogens and the occurrence of endometrial cancers. To reduce this risk, natural progesterone or synthetic progesterone (progestin) is used to stimulate atrophy or regression of endometrial lesions[4-7]. Nevertheless, on account of the short biological half-life of natural progesterone, its use is limited**;** therefore, progestins are widely used by women during MHT treatment[8-10]. However, the role of progestin in increasing breast cancer risk was reported by multiple clinical studies such as the Million Women Study (MWS) and Women's Health Initiative (WHI). They have demonstrated that combined estrogen-progestin therapy is associated with an increased risk of developing breast cancer[11,12]. Various progestins can exert different effects on breast cancer cell proliferation. Among the 9 selected types of natural progesterone and progestins, NET is the most potent progestin for promoting breast cancer cell proliferation[13]. In addition, we found that NET stimulated breast cancer cell viability of MCF-7 (ER+/PR+/HER2-) and T-47D (ER+/PR+/HER2-) in a dose-dependent manner[1].

PGRMC1 Mediates Progestin-Stimulated Effect on Breast Cancer

PGRMC1 is approximately a 25 kDa protein. As a non-classical membrane localized progesterone receptor, PGRMC1 binds progestogen with medium to high affinity[14-16]. Compelling evidence has accumulated showing that PGRMC1 contributes to cancer progression. Firstly, its expression was found up-regulated in tumors of the breast, thyroid, colon, ovary, cervix, and lung[17-21]. Secondly, PGRMC1 exhibited several cellular functions involved in the progression of several cancers. For example, PGRMC1 was found to be able to promote mitosis in spontaneously immortalized granulosa cells and human ovarian cancer cells[22], and to increase cell proliferation and migration of lung cancer cells as well[23]. Most interestingly, PGRMC1 mediated anti-apoptotic effect of progesterone in rat granulosa cells and in the immature rat ovary; this effect may be produced through interacting with plasminogen activator inhibitor RNA-binding protein-1[14,24-26]. Depletion of PGRMC1 completely abrogated the anti-apoptosis effect of progesterone[15,19]. Furthermore, overexpression of PGRMC1 decreased the apoptosis rate in spontaneously immortalized granulosa cells[26,27]. In addition, a study indicated that PGRMC1 inhibitor abrogated the inhibiting effect of progesterone on proteins ratio of BAX/BCL-2 in neurons[28], and overexpression of PGRMC1 elevated BCL-2 expression in ovarian cancer cells[29]. Another independent study indicated that the underlying mechanism of the anti-apoptotic effect of PGRMC1 is exerted through regulating expression of BH-3 only protein-Harakiri, a BCL-2 interaction protein[30]. Of note, BCL-2 functions as an oncogenic protein by inhibiting cell apoptosis. Overexpression of BCL-2 is common in breast cancer, especially in estrogen receptor (ER)-positive breast tumors[31].

PGRMC1 is widely expressed in mammalian tissues and immortal mammalian cell lines. During the last 10 years, we have made contributions to elucidating the role of PGRMC1 in promoting breast cancer through hormone therapy. *In vitro* experiments showed that progestins can stimulate a proliferative response in PGRMC1-overexpressing MCF-7 cells[13], and *in vivo* NET-promoting tumor growth was observed in the xenografts bearing tumors developed from PGRMC1-overexpressed breast cancer cell T-47D[32]. Moreover, our clinical sample analysis revealed an increased expression of PGRMC1 in breast cancer tissues compared with surrounding normal tissues. The increased levels of PGRMC1 were positively correlated with aggressive phenotypes and poor prognosis[33,34]. All these studies suggest that PGRMC1 plays an important role in the progression of breast cancer, especially during hormone therapy. Along with these findings, our recent publication elucidated that NET markedly increases PGRMC1 expression in human breast cancer MCF-7 cells[1].

Anti-tumor effect of miRNA

MiRNAs are a group of endogenous small non-coding regulatory RNAs, 21 to 24-nt in length. They silence their targeted genes at the post-transcriptional level. Usually, miRNAs interact with the targeted mRNAs within the 3′ untranslated regions (UTR) of target genes[35]. Aberrant expression of different miRNAs has been demonstrated in all kinds of cancer phenotypes[36-38].

Our lab has been working on the pathophysiological roles of lymphocyte-derived microparticles (LMPs) for over ten years. LMPs were produced from human CEM T cells with treatment of actinomycin D[39,40]. We have strong evidence that LMPs play an important role in suppressing pathological angiogenesis and tumor progression[41-46]. In order to dissect the active components of LMPs, we performed miRNA sequence analysis and revealed that miR-181a is one of the most plentiful miRNAs in LMPs. Several recent studies have suggested that miR-181a expression was downregulated in some types of human tumors such as lung cancer, oral squamous cell carcinoma, glioma etc.[47-52]. Nevertheless, other studies indicated that miR-181a may perform oncomir activities by facilitating the metastasis and invasion of cancers[53-55]. Although contradictory data were reported, multiple clinical studies suggested that miR-181a expression was negatively correlated with breast cancer in late stages, with worse prognosis and with aggressive phenotypes[54,56-58]. The hypothesis that miR-181a may function as an anti-oncogene is further supported by

our recent *in vitro* studies. We transfected the miR-181a-5p mimic into breast cancer cells and noticed that transitory overexpression of miR-181a significantly reduced cell viability of MCF-7 and T-47D breast cancer cell lines in a dose-dependent manner[1].

MiR-181a Inhibits Progestin-Stimulated Breast Cancer

The mechanisms underlying the anti-tumor growth effect of miR-181a during NET treatment were investigated by our research group. We found that the apoptosis rate of the MCF-7 breast cancer cells in the miR-181a overexpression group was dramatically increased. Consistent with these results, we found a significantly decreased expression of the anti-apoptotic gene BCL-2 and a dramatic increase of the pro-apoptotic gene BAX and Caspase 9 in MCF-7 cells transfected with miR-181a. These results indicated a pro-apoptotic role of miR-181a involved in the anti-tumor growth effect. In addition to decreasing cell viability of MCF-7, miR-181a also strongly abrogated the NET-induced cell viability of MCF-7 cells by increasing apoptosis[1].

Apoptosis is a form of regulated cell death that is governed by pro- and anti-apoptotic members of the BCL-2 protein family[59]. Lack of apoptosis can trigger oncogenic transformation at different stages and lead to increased tumor growth and survival during the metastatic process and chemotherapy. Increased expression of the anti-apoptotic gene BCL-2 is found in many types of cancer. However, upregulation of BCL-2 expression can occur through all kinds of mechanisms. Therefore, preventing anti-apoptotic gene BCL-2 expression seems primordial in treating certain types of cancer. Activation of BCL-2-associated X protein (BAX) results in mitochondrial outer membrane permeabilization, which is a necessary step for releasing small hemeprotein to activate the Caspase 3 and Caspase 7[60]. Because the MCF-7 cell lacks functional Caspase 3[61], mitochondrial-dependent apoptosis proceeds via Caspase 7 and Caspase 9 activation. This is in keeping with an increase of Caspase 9 in miR-181a treated human breast cancer cells[1]. We have shown that miR-181a downregulated the expression of apoptosis related gene expression. Among these genes, BCL-2 is a direct target of miR-181a, which has been verified by luciferase assay in breast cancer cells[62]. In addition, PGRMC1 expression was decreased in the miR-181a overexpressing breast cancer cells. Furthermore, miR-181a attenuated NET-stimulated PGRMC1 expression in progesterone receptor positive and negative breast cancer cells[1], which indicates that miR-181a probably exerts a pro-apoptotic effect through downregulation of PGRMC1 in these cells. These data lead us to assume the existence of functional interactions between PGRMC1 and miR-181a, although it has not been shown that they have a direct physical interaction[63]. As we mentioned above, since BCL-2 is a possible downstream effector of the anti-apoptotic action of PGRMC1[28-30], it is reasonable to speculate that the mechanism of miR-181a increasing apoptosis of breast cancer cells is due to decreased expressions of BCL-2 and PGRMC1.

Application Prospect of miR-181a as an Anti-Tumor Therapy

In brief, miR-181a exerts strong anti-breast cancer activity, and this activity occurs through regulating the expression of apoptosis-related genes and PGRMC1. There is growing evidence that PGRMC1 contributes to cancer pathology. Up-regulated expression of PGRMC1 was found in a variety of tumors[17-21]. PGRMC1 facilitates the degradation of doxorubicin through its ability to bind CyP450[20], which paves the way for speculation that a similar effect may be involved in the PGRMC1 mediated resistance to chemotherapeutics. In addition, overexpression of PGRMC1 can also lead to increased cancer cell proliferation and metastasis[20]. All these data suggest that PGRMC1 is of great importance to cancer progression. For the first time, we demonstrated that miR-181a can reduce PGRMC1 expression. Thus, miR-181a may develop into a novel therapeutic strategy not only for breast cancer treatment but also for other types of cancer overexpressing PGRMC1.

We have reported that miR-181a attenuates MCF-7 and MDA-MB-231 cell viability by reducing the upregulated protein expression of PGRMC1 induced by NET[1]. However, there are many other kinds of progestins which are widely used during hormone therapy. For instance, medroxyprogesterone acetate, which can also lead to the development of breast tumors *in vivo* and increase lymph node metastasis[64]. Further research could focus on the role miR-181a plays in reducing breast cancer cell viability provoked by different kinds of progestins. The aim would be to explore whether miR-181a has an extensive inhibitory effect on hormone-induced breast cancer.

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