The role of nitric oxide in endometrial carcinoma

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Endometrial carcinoma is the sixth most common type of cancer in women. The constant increase in incidence is probably related to the increasing prevalence of obesity. Nitric oxide as a signaling molecule is involved in several diseases, including cancer. It is an important vasodilator, it inhibits platelet aggregation and it is important in the immune response. A key mediator of immune activation and inflammation is inducible nitric oxide synthase (iNOS) which has a dual role depending on the position and localization of nitric oxide.
Key words: endometrial carcinoma, nitric oxide, inducible NOS, cyclooxygenase-2

Úloha oxidu dusnatého pri karcinóme endometria
Karcinóm endometria je šiestym najčastejším druhom zhubného ochorenia u žien. Stále narastanie incidencie pravdepodobne súvisí so zvyšujúcou sa prevalenciou obezity. Oxid dusnatý ako signálna molekula sa podieľa na viacerých ochoreniach vrátane rakoviny. Je dôležitým vazodilatátorom, inhibuje agregáciu krvných doštičiek a má význam pri imunitnej odpovedi. Kľúčovým mediátorom imunitnej aktivácie a zápalu je indukovateľná syntáza oxidu dusnatého (iNOS), ktorá má dvojitú úlohu v závislosti od koncentrácie a lokalizácie oxidu dusnatého.
Kľúčové slová: karcinóm endometria, oxid dusnatý, induktívna NOS, cyklooxygenáza-2

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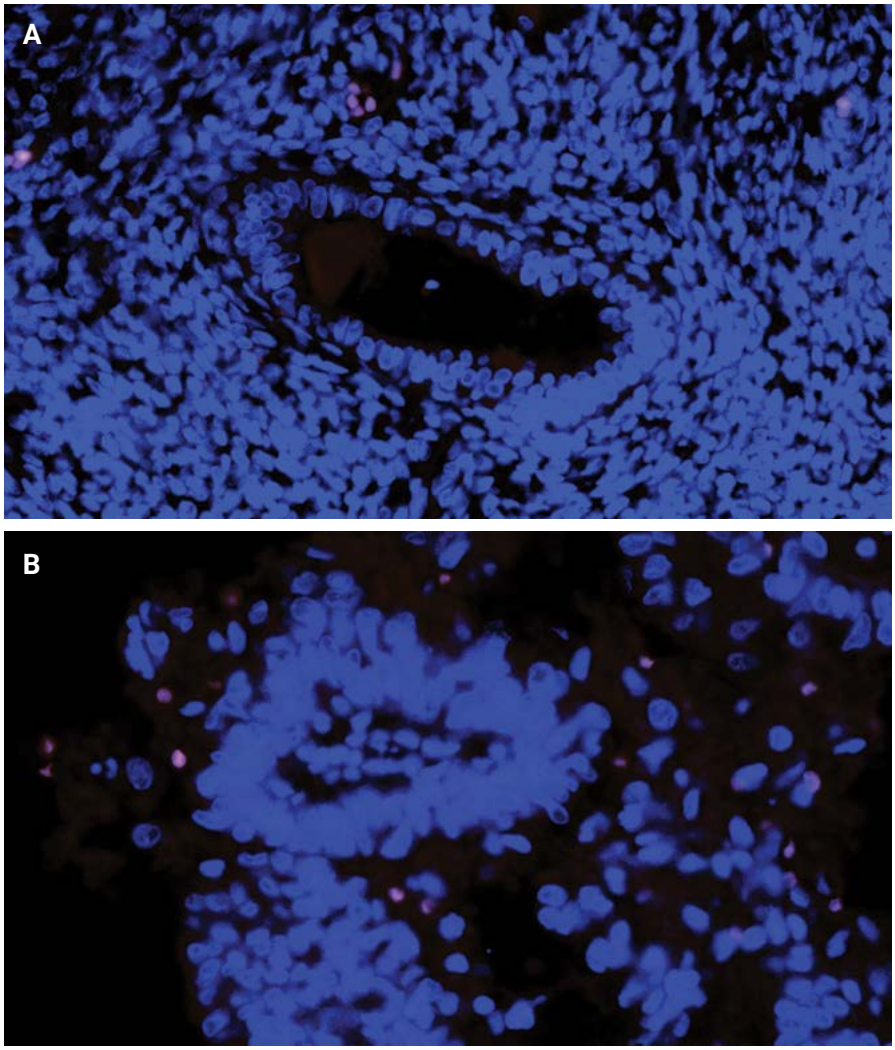
Introduction

Endometrial carcinoma is the most common type of cancer of the female genital tract in developed countries. Worldwide, it is the sixth most common malignant disease in women[1]. The incidence is still increasing, probably due to the increasing prevalence of obesity. It is manifested by abnormal vaginal bleeding, which complicates the detection of cancer in premenopausal women. In these women the disease is usually detected in later stages. However, the incidence of endometrial cancer is greater in postmenopausal women, in whom early detection is associated with good prognosis. The currently accepted model of carcinogenesis categorizes endometrial carcinoma into two broad classifications: type I or type II based on genetic and morphological features[2]. The endometrium naturally undergoes structural modifications and changes in specialized cells in response to fluctuations in estrogen and progesterone levels during the menstrual cycle. Prolonged exposure of cells to high estrogen levels leads to hyperplasia of endometrial cells, which increases the risk of developing atypical hyperplasia, a thickening of the endometrium which is a precursor of type I endometrial cancer. Type I is generally associated with elevated estrogen levels that are not suppressed by progesterone[3]. It occurs more often (85 %) than type II, it has a higher survival rate and lower rate of recurrence[4]. It is often diagnosed in the early stages and therefore has a better prognosis. Risk factors include those that lead to increased estrogen levels without increasing protective progesterone levels. These include obesity, in which increased adiposity increases aromatase activity, leading to the conversion of androgenes to estrogenes; treatment with tamoxifen, which blocks estrogen receptors in the breasts but stimulates them in the uterus; postmenopausal estrogen therapy without progesterone; estrogen-producing tumors; nulliparity; chronic a anovulation and last but not least aging[5]. Excessive fat consumption and being overweight (defined as a BMI of at least 25 kg/m^2) is a major risk factor present in almost 50 % of women with endometrial carcinoma. BMI over 25 kg/m^2 doubles the risk of developing endometrial cancer and BMI over 30 kg/m^2 even triples the risk[6]. Obesity remains the risk factor even when circulating estrogen levels are normal[7]. On the other hand, administration of hormonal contraceptions or multiple births have an effect on reduction the risk of endometrial carcinoma. Type II endometrial cancer is more rare than type I and isn't linked to estrogen levels. However, it is more aggressive, manifests itself in advanced stages and therefore has worse prognosis. It affects women who have endometrial atrophy, lower body weight and develop later in life. It is also more common in women of african descent[8].

Characteristics, properties and signaling of nitric oxide

Nitric oxide (NO) is a signaling molecule involved in several diseases including cancer[9]. It is a potent vasodilator, inhibitor of platelet aggregation, neurotransmitter and also important in the immune response. It is synthesized endogenously in several tissues by converting L-arginine to L-citruline in the presence of four isoforms of nitric oxide synthase (NOS). Endothelial and neuronal NOS are expressed constitutively and they are calcium-dependent and produce NO in low concentration in pico- and nanomolar range over a short period of time ranging from a few seconds to minutes to regulate various signaling pathways. Inducible NOS (iNOS) is calcium-independent and it is induced by cytokines, endoto-

Figure 1. nNOS expression in endometrium. nNOS did not show striking positivity neither in physiological endometrial tissue (A) nor in cases of endometroid endometrial carcinoma (B). nNOS Ms Ab, DyLight 594, 400x



xines and hypoxia under oxidative stress. On the contrary, it produces significant concentrations of NO (in the micromolar range) over a longer period of time ranging from hours to days. Mitochondrial NOS (mNOS) is considered as an alternative to neuronal NOS (nNOS) which is produced in mitochondria[10]. NOS is expressed differently in obese and nonobese individuals and it is overexpressed in many tumors[11]. In cancer, NO acts in two ways depending on the concentration. At pico- and nanomolar concentrations it promotes tumorigenesis by activation of angiogenesis, which stimulates tumor progression by allowing oxygen and nutrients to flow into the tumor, leading to cell proliferation. It also promotes invasiveness and the formation of metastases[12]. However, at high concentrations (micro- to millimolar) it has anti-cancer effects, causing extensive DNA damage, oxidative and nitrosative stress, which leads to cytotoxicyty and apoptosis of tumor cells[13].

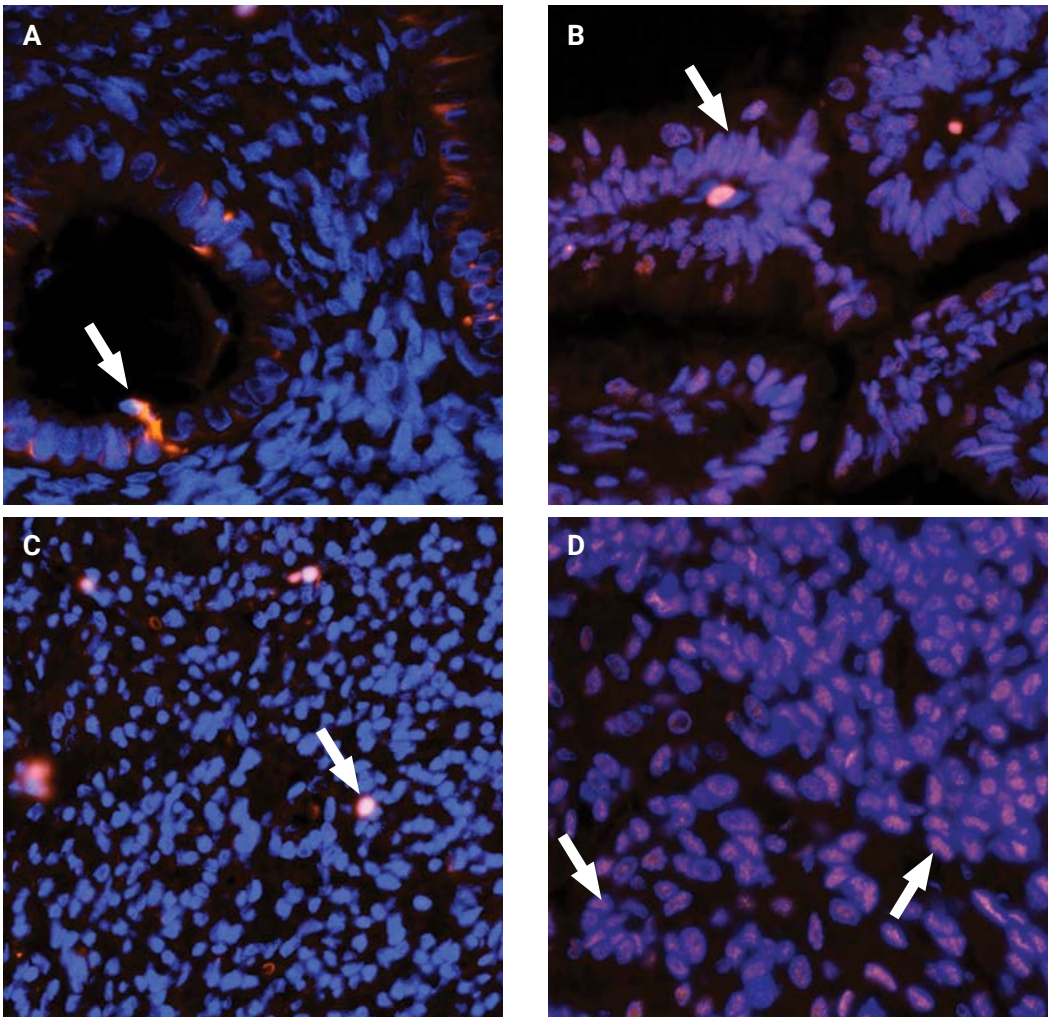
The presence of NOS in the endometrium has been described in many species, suggesting a role of NO in normal endometrial functions. In the human endometrium, endothelial (eNOS) and iNOS have been localized to the glandular epithelium in the nonpregnant uterus. Likewise, NO may be involved in the initiation and control of menstrual bleeding. In addition, it may play a role in inhibiting platelet aggregation in the endometrium, where menstrual hemostasis is thought to occur primarily through vasoconstriction rather than clot formation[5].

Our analysis (**Figure 1-3**) shows that regular expression of eNOS is predominant in endometrial tissue, particularly in endometrial glandular epithelial cells. iNOS shows only focal irregular positivity in epithelial cells as well as in stroma. In cases of endometrial carcinoma, we observed a striking increase in nuclear positivity of iNOS in both tumor cells and stroma.

Angiogenesis and iNOS

Angiogenesis, the process that leads to the formation of new blood vessels, is a prerequisite for tumor growth due to the need of oxygen and nutrients. Molecular studies have shown that susceptibility to gynecological tumors such as endometrial carcinoma and cervical cancer increases due to increased inflammatory markers including increased expression of iNOS and cyclooxygenase-2 (COX-2)[14]. COX-2 is a key enzyme in the biosynthesis of prostaglandins and it is involved in the development of inflammatory processes and carcinogenesis, including the induction of angiogene-

Figure 2. *iNOS expression in endometrium. iNOS exhibited focal cytoplasmic positivity in both sporadic endometrial glandular cells (A) and stroma (C) in physiological endometrial tissue. However, there was a striking increase in nuclear positivity of iNOS in cases of endometrial endometrioid carcinoma (B, D). iNOS Ms Ab, DyLight 594, 400x*

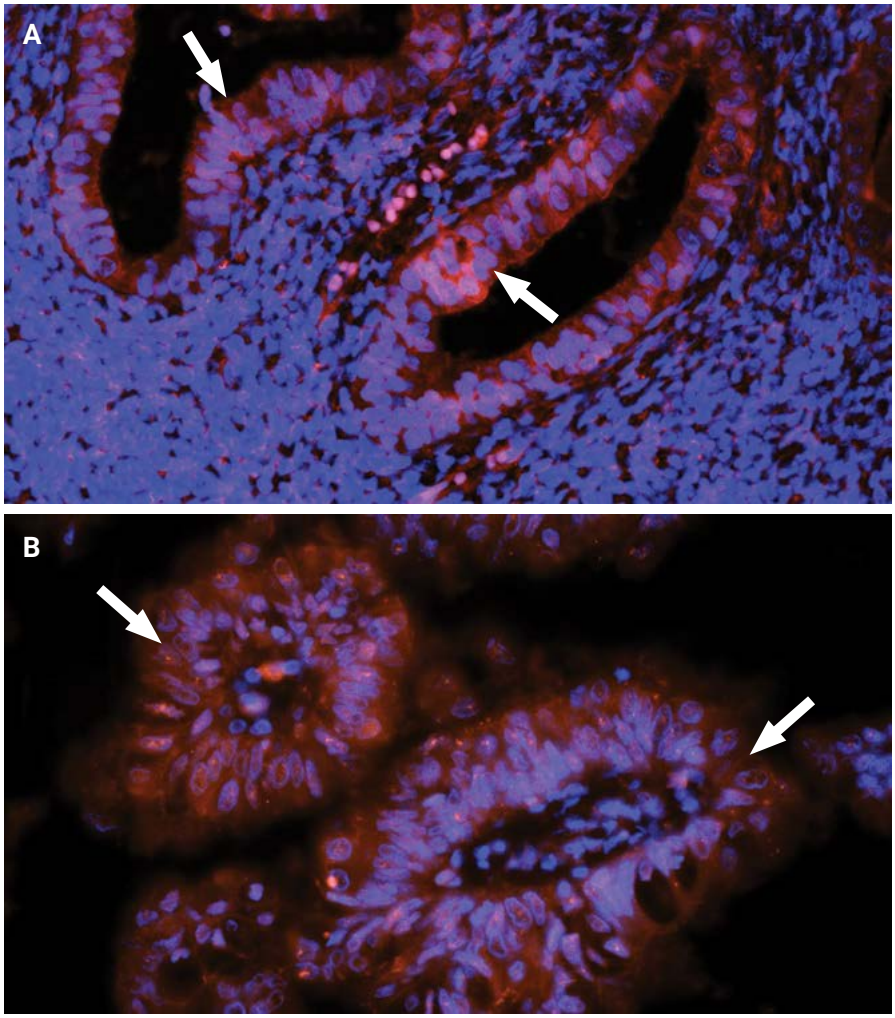


sis. Immunohistochemical analysis also revealed that COX-2 expression was significantly associated with endometrial cancer risk and development. Increased production of prostaglandins and increased release of COX-2 can induce neovascularization[15]. However, the prognostic significance of COX-2 expression in endometrial carcinoma remains controversial. Recently, experimental studies have shown that NO produced by iNOS increases COX-2 activity. Li et al.[16] in their study investigated the significance of COX-2 and iNOS in thirty woman patients with endometrial carcinoma. They investigated the relationship of these molecular markers to tumor characteristics and microvascular density. They showed that iNOS overexpression in endometrial carcinoma significantly correlated with microvascular density. In this study, expression of iNOS was detected in 21 of 30 (73 %) tumors but in none of the five normal endometrial samples. Microvascular density was higher in patients with confirmed iNOS expression than in those without iNOS expression. Thus, iNOS expression is significantly correlated with microvascular density. These researchers also confirmed that endometrial cancer cells are able to modulate NO synthesis. Their results show that a significant up-regulation of iNOS in endometrial cancer cells may be responsible for endometrial tumor carcinogenesis. The association between iNOS overexpression and tumor spread could be supported by iNOS- mediated increased ability of invasion. On the other hand, several studies show that the presence of NO in tumors or their microcirculation has adverse affects on survival of malignant cells[17]. Its positive effect can be mediated through the mutagenesis of p53, subsequently leading to the loss of its inhibitory effect and the up-regulation of Bcl-2 expression which has antiapoptotic effects[18]. Typical NO mechanisms mediating apoptosis include caspase activation, chromatin condensation and DNA fragmentation[19]. NO can inhibit DNA synthesis by hypoxia or by regulation of the expression of p53 and other apoptosis-related proteins, which inhibit tumor growth or kill tumor cells through cytotoxicity[20].

Conclusion

NO plays an important role in tumor initiation, growth and metastasis in endometrial carcinoma. However, the inducible isoform of NOS appears to play a dual role in cancer that depends on the concentration and localization of NO. In cancer cells, it can contribute to increased proliferation, metastasis and even drug resistance. On the other hand, it can adversely affect the survival of tumor cells.

Figure 3. eNOS expression in endometrium. eNOS shows strong and regular cytoplasmic expression both in cases of physiological endometrial tissue (A) and in cases of endometrial endometrioid carcinoma (B). eNOS Rb Ab, DyLight 594, 400x



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