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Histochemical evaluation of angiogenesis in endometrial adenocarcinoma

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Summary

The formation of new vessels from endothelial cells, termed angiogenesis, is a complex process that is controlled by numerous paracrine factors. Vacularization of tumor tissue is a prerequsite for tumor growth and enables the dissemination of tumor cells throughout the body. Angiogenesis is classically assessed by counting the vessels or clusters of endothelial cells after selective immunohistochemical staining. In this study, vascularization of tumor tissue was evaluated quantitatively in 36 cases of stage I endometrial adenocarcinoma. The goal of this study was to evaluate whether the microvessel density correlates with the invasion potential of endometrial adenocarcinoma.

The results show an increase in the number of endothelial cells at different stages in endometrial adenocarcinoma stage I and a strong positive correlation between the endothelial-to-stromal ratio and tumor grading.

Introduction

The formation of new peri- and intratumoral blood vessels is one of the essential events in tumor growth and a prerequsite for formation and dissemination of metastasis (Abulafia and Sherer,

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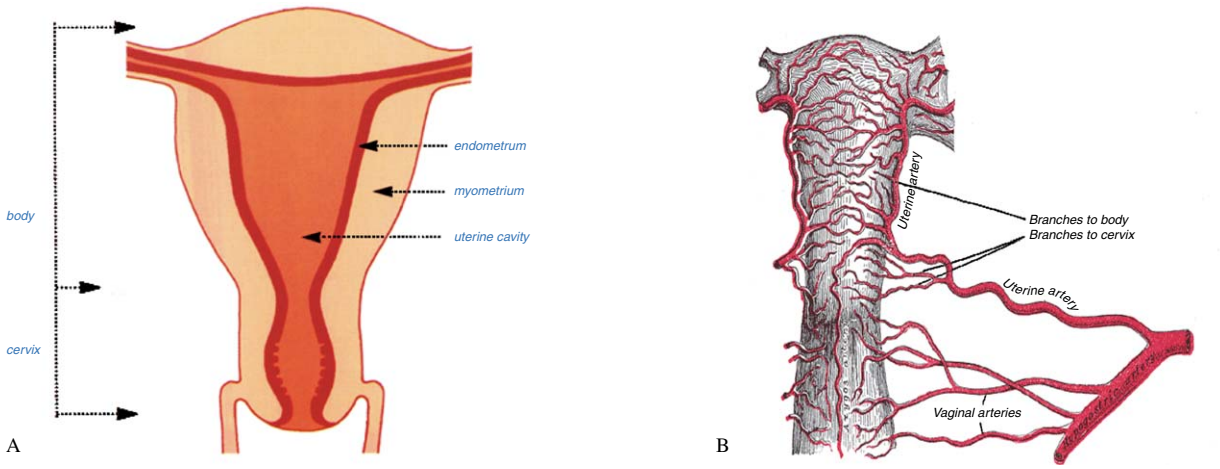

Figure 1. Structure and vascularization of the uterus (After Williams, 1995). (A) parts and layers of the uterus and (B) vascularization of the body and cervix of the uterus.

1999; Folkman, 1992; Kerbel, 2000; Gupta and Qin, 2003). The extent of vascularization is considered to be a marker of tumor aggressivity and a significant prognostic factor (Homesley and Zaino, 1994; Weidner, 1995). We quantitatively studied microvascular density in uterine adenocarcinoma at different stages, using immunohistochemistry (Fig. 1).

Modern methods of quantitative morphology (Chaplain et al., 1995) have been used to determine the peri- and intratumoral microvessel density on immunohistochemically stained slides. The tumoral endothelial cells can be selectively stained by using various markers (e.g. von Willebrand, CD34, CD31) (Yaegashi et al., 1995). The prognostic value of these analyses has often been discussed in the literature with respect to risk of tumoral spread (Kirschner et al., 1996; Hanhahan and Folkman, 1996; Salvesen et al., 1998; Obermair et al., 1999).

Material and methods

In this study, hysterectomy specimens collected from 36 patients with stage I uterine adenocarcinoma were used. The specimens were fixed in formalin and paraffin-embedded. Slides were immunohistochemically stained for CD34 and analyzed with an Olympus optic microscope. Images were digitally processed with a professional software (ImageProView). Endothelial-to-stromal ratio was automatically calculated in the "hot spot" areas with highest microvessel density, according to Weidner's method (Weidner, 1995).

Results

In endometrial adenocarcinoma stage I specimens that have been immunohistochemical stained for CD34 the microscopic aspects are different according to tumoral grade (De Vita, 1997).

(a) In the *well-differentiated* endometrioid adenocarcinoma, the tumoral tissue is arranged in tubular glands resembling those of normal proliferative endometrium (Spiegel, 1995; Noci et al., 1996). However, these glandular structures are crowded and convoluted and exhibit an irregular arrangement. Epithelial cells lining

Figure 2. (a) Stromal vessels immunohistochemically stained for CD34 in a well-differentiated stage I endometrial adenocarcinoma (magnitude $\times 100$), (b) detail of compressed endometrial stroma with positive immunohistochemical staining for CD34 of endothelial cells (magnitude $\times 400$), (c) positive immunohistochemical staining for CD 34 in tumoral stroma (magnitude $\times 100$), (d) magnified image of tumoral stroma with positive immunohistochemical staining for CD 34 in endothelial cells at the endometrial–myometrial junction (magnitude $\times 400$), (e) numerous vessels positively stained for CD34 in the invasion area into the myometrium ($\times 400$), (f) endometrial adenocarcinoma with high microvascular density in the area of invasion toward the cervix (in the arrow – an invaded endocervical gland) ($\times 100$), (g) endothelial cells with intense immunohistochemical staining for CD34 in the invasion area towards the myometrium – mild-differentiated endometrial adenocarcinoma ($\times 100$), (h) mild-differentiated endometrial adenocarcinoma – magnified image of tumoral vessels ($\times 400$), (i) mild-differentiated endometrial adenocarcinoma (in the arrow – a capillary vessel with tumoral embolus inside) ($\times 400$) and (j) thin stromal axes with intense staining for CD34 in poorly differentiated endometrial adenocarcinoma ($\times 400$).