ANGIOGENESIS IN BREAST CARCINOMAS WITH DIFFERENT EXPRESSION PROFILES

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ABSTRACT

Endoglin is a glycoprotein preferentially expressed in proliferating vessels during angiogenesis. It is described that high angiogenic index detected by endoglin is associated with poor prognosis in breast cancer patients. This indicates that endoglin could serve as a target for antiangiogenic therapy. However, the relationship between angiogenic index and the molecular subtypes of breast carcinoma is not yet extensively explored, especially in the basal-like subset, in which the anti-angiogenic therapy could be an option, since these carcinomas still do not have a specific therapy.

We have studied the expression of endoglin on formalin-fixed paraffin-embedded tissue sections of invasive breast carcinomas by using immunohistochemical assays in a series of 161 patients.

Our results show that although the angiogenic index is higher in the basal-like subtype than in the other groups of breast carcinomas, this difference is not statistically significant. This suggests that the use of an antiangiogenic therapy can be valid in all the subtypes of breast cancer, as combined therapy with chemotherapeutic agents.

INTRODUCTION

Angiogenesis is a complex multistep process required for tumour growth and metastasis. It involves endothelial cell migration and proliferation, microvessel differentiation and anastomosis, and extracellular matrix remodelling[2].

Studies have shown that endoglin (CD105) is involved in the development of blood vessels and that it represents a specific marker of neovascularization for several types of tumours[4]. Endoglin is a cell-surface glycoprotein recently identified as an optimal indicator of human endothelial cells proliferation. Furthermore, the angiogenic index detected by its expression has been correlated with poor prognosis in breast cancer patients[3]. Collectively, these data suggest that the tumour microvasculature may constitute a relevant target for antiangiogenic therapy. Specifically, endoglin, because of its preferential expression in the newly formed endothelium, can be considered a potential target for this kind of treatment. Despite these evidences, the relationship between angiogenesis, assessed by the immunohistochemical expression of endoglin, and the molecular subtypes of breast carcinomas has not yet been addressed.

Gene expression profiling has classified invasive breast carcinomas into different subtypes[6], based on the expression of two molecular markers: ER and HER2. Thus, tumours can be classified as: Luminal A type (ER+/HER2-), Luminal B (ER+/HER2+), basallike (ER-/HER2-) or HER2-overexpressing carcinomas (ER-/HER2+). Basal-like breast carcinomas are also characterised by expression of basal/myoepithelial cell markers, such as CK5, P-cadherin, EGFR and p63 (5). Unlike estrogen receptor positive cancers, that respond well to hormonal therapy and HER2-overexpressing tumours that are responsive to Trastuzumab, basal-like carcinomas do not have a specific therapy and display a preferential hematogenic pattern of metastasis.

The aim of the present study was to evaluate the relationship between angiogenesis, assessed by the immunohistochemical detection of endoglin and the molecular subtypes of human invasive breast carcinomas, defined by microarray gene expression profiling and validated by immunophenotype. Also, we would like to clarify if basal-like carcinomas have a higher angiogenic index and whether there is evidence to indicate that endoglin could be a potential target for antiangiogenic therapy, especially in the basal-like subset of breast carcinomas.

METHODOLOGY

We have studied a cohort of 161 cases of invasive breast carcinomas, collected from the archives of the Pathology Department of the Federal University of Santa Catarina, Florianópilis, Santa Catarina, Brazil.

Immunohistochemical staining for endoglin (CD105) in two-micron thick sections of whole tissue was performed using the streptavidin-biotin-peroxidase method. Antigen unmasking was carried out using a commercially available solution of citrate buffer pH=6,0 (Vector Laboratories, Burlingame, CA, USA) at 98ºC for 30 minutes. The slides were incubated with the primary antibody anti-CD105, clone 4G11 (Novocastra, UK) in a 1:50 dilution overnight at 4ºC and a DAB solution (3,3-diaminobenzidinetetrahydrochloride) (DakoCytomation, Carpinteria, CA, USA) was used as a chromogen. Paraffin sections of a breast invasive carcinoma were used as positive controls in every run.

The samples had been previously tested for ER, HER2, CK5, EGFR, P-cadherin and p63 status in Tissue Microarrays.

We used the Microvessel Density to assess the expression of endoglin. For that, we chose 3 hot spots areas in each sample and counted the stained blood vessels in the observational field of 200x magnification, as previously described[1]. Then, we calculated the mean value of stained vessels per case. The X^2 contingency test was used for cate-

gorical variables to determine associations between groups (the various subtypes of breast tumours and the expression of endoglin). A p value < 0,05 was considered to represent a significant difference.

RESULTS, DISCUSSION AND CONCLUSIONS

Endoglin was expressed in the vascular endothelial cells in almost all cases. The staining was observed in the endothelial cell membrane and cytoplasm of newly-formed vessels (Figure 1).

Then, we grouped the cases according to their immunohistochemical profile for ER, HER2 and basal markers and calculated the mean value of stained vessels per tumour subtype.

We demonstrated that the angiogenic index measured by endoglin was similar in all subtypes of breast carcinomas (Table I and Figure 2).

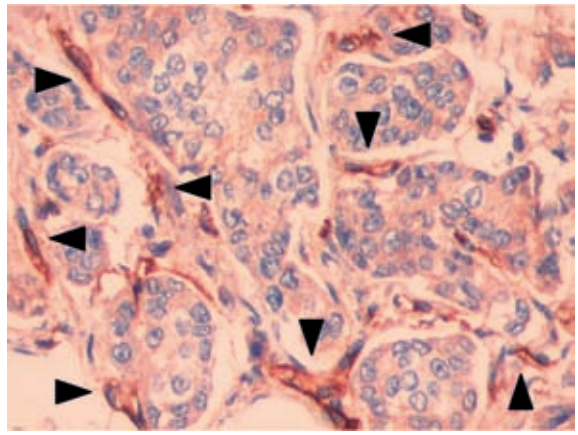

Figure 1 – Endoglin immunohistochemical staining of newly-formed blood vessels (arrows) in an invasive breast carcinoma (magnification of 400X)

The number of stained vessels was higher in the basal-like subgroup (the mean value is 28,8 microvessels per mm^2), in comparison with the other molecular subtypes (27,3 microvessels per

Table I – Expression of endoglin in the distinct breast tumour molecular subtypes

Tumour subtype	Stained vessels (mean values per mm^2)	Standard deviation
Luminal A	24,9	11,4
Luminal B	18,6	3,8
Basal-like	28,8	9,7
HER2-overexpressing	27,3	10,3

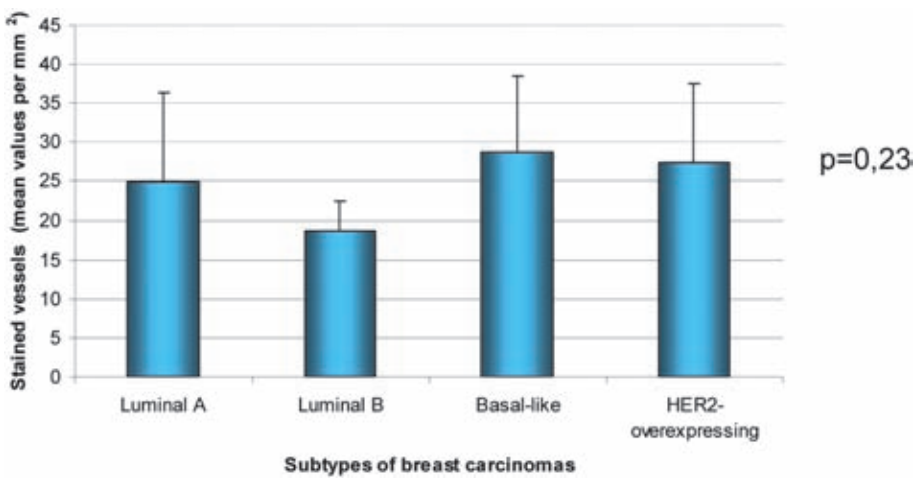

Figure 2 – Expression of endoglin in the various breast tumour molecular subtypes

mm^2 in HER2 overexpressing carcinomas, 24,9 microvessels per mm^2 and 18,6 microvessels per mm^2 for luminal A and luminal B, respectively). However, these differences are not statistically significant (p=0,23). This suggests that endoglin is not a good discriminator of the different subtypes of breast cancer.

Although these results should be validated in different and larger series, they show that basal-like breast carcinomas have a similar angiogenic index when compared to the other subtypes, and that an antiangiogenic therapy can not be claimed as a specific therapy to this subset of breast carcinomas, which does not avoid its use in combination with chemotherapy for all the subsets of breast carcinomas.

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