Angiopoietins 1, 2 expression by infiltrating stroma in to MLS, ovarian carcinoma tumors lead to heterogeneity in vascular maturation and permeability

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Introduction

Tumor angiogenesis is characterized by high structural and functional heterogeneity. Angiopoietins (Ang-1 and Ang-2) collaborate with VEGF in regulation of vascular remodeling and recruitment of peri-endothelial cells (vascular maturation). In addition, Ang-1 blocks VEGF induced plasma leakage without altering vessel morphology. Ang-2, the Ang-1 antagonist, induces destabilization of mature vessels[1]. The aim of this study was to explore the relationship between tumor vessel architecture, permeability and maturation, in the context of VEGF and angiopoietins expression in MLS (human ovarian carcinoma) tumors.

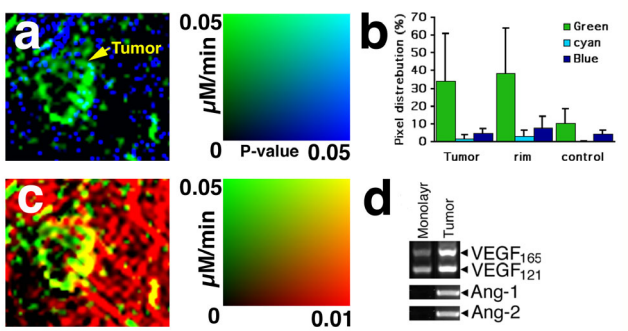
Materials and methods

Tumors were generated by inoculation of 0.5-1x10^6 MLS, ovarian epithelial carcinoma cells to the lower back of CD-1 nude mice (females, 6 weeks old). MRI experiments were performed on a horizontal 4.7 T Bruker Biospec using rf decoupled 1.5cm surface coil (slice thickness 1 mm, SW 50,000 Hz, FOV 3.5 cm). *Vascular maturation maps* were generated using pixel by pixel t-test from change in MR SI in response to hypercapnia[2] (Gradient echo, flip angle of 40; TR 230 ms, TE 10 ms; 256x256 pixels; 117 s/image). *Maps of fPV and APS*: using CM, biotin-BSA-Gd-DTPA (12 mg/mouse in 0.2 ml). (Spin echo, TRs 100 200 500 1000 ms, TE 10.6 ms, matrix 128X128 zero-filled to 256x256, 52 s/image)[3]. *Histology:* paraffin sections were stained with α-SMA and avidin-FITC (for biotinylated BSA) [4] and DAPI. *RT-PCR:* Total RNA was isolated and reverse transcribed. PCR with primers for: huVEGF, human/mouse Ang-1 and Ang-2.

Results and discussion

In order to study the relationship between plasma leakage and vascular maturation, DCE-MRI was combined with BOLD contrast MRI. Plasma leakage (APS- apparent permeability surface product) was monitored using biotin-BSA-Gd-DTPA as contrast material (CM)[3]. Correlation maps (fig. a) of maturation (blue) and permeability (APS; green) showed independence between the two processes. Quantitative analysis of these maps (fig. b, n=5) showed increased permeability in the tumor and the tumor margin ($P<0.1$ and $P<0.05$ respectively, 2-tailed paired, ttest). In this analysis three different vessel populations were detected in the tumor region: (1) mature non-leaky (blue), (2) mature leaky (cyan) and (3) immature leaky vessels (green), suggesting that the molecular regulation of maturation and permeability is not uniform across the tumor. Co-staining with α-SMA and the biotinylated CM[4] (with avidin-FITC) showed CM extravasation mostly in the tumor periphery but also along stroma tracks inside the tumor. Plasma leakage was only occasionally observed in association with mature vessels. In other regions α-SMA positive track and vessels showed poor staining for the CM, indicating low permeability. Rarely, α-SMA positive vessels appeared empty of the CM, suggesting that these could be non-functional or regressing vessels. The histological observations, which are consistent with the MRI findings, suggest independent regulation of vascular maturation and permeability.

In order to understand the impaired regulation of vascular maturation and permeability at the molecular level, VEGF, Ang-1 and 2 expression was evaluated by RT-PCR in MLS cell line in vitro and in vivo. Two human VEGF isoforms (165 and 121) were detected in cells monolayer, multicellular spheroids and MLS tumors inoculated in to CD-1 nude mice (n=12). However, Ang-1 and Ang-2 were detected only in MLS tumors (n=12/12 and 9/12 respectively), but not in culture (fig. d). Moreover, tumor derived angiopoietins showed complete homology to the mouse but not human genes, suggesting expression by the host infiltrating stroma cells and not by the tumor cells. Invasion of stroma cells was confirmed by histology. Invading stroma cells were α-SMA positive suggesting that these are peri-endothelial cells or myofibroblasts, which envelop blood vessels.



In order to evaluate tumor effect on angiopoietins expression by the host tissue, RT-PCR analysis was applied. Expression of Ang-1 was detected in only 1 out of 7 normal skin samples. However, expression of Ang-1 was detected in most of the skin samples at the site of intradermal injection of VEGF 165 after 4, 8 and 16 hr (69%, n=13). Surprisingly, Ang-1 was detected also in control samples 4 hr and 16 hr after intradermal injection of PBS (75%, n=8). In contrast to Ang-1, Ang-2 was not expressed in the skin in the absence of tumors, neither in intact mouse skin, nor after intradermal injection of PBS (n=8) and was rarely detected after intradermal injection of VEGF 165 (15%, n=13). These finding suggest that both ang1 and 2 upregulated by chronic interference (tumor) while only ang-1 is upregulated by acute interference (wound) to the tissue integrity.

The fraction of plasma volume (fPV) and the APS also were studied by DCE-MRI using biotin-BSA-Gd-DTPA as contrast material. Correlation of the two maps and quantitative data analysis (fig. c; n=5) revealed that high APS (green) was frequently associated with low fPV (red), and vice versa. Presumably, regions with low fPV are more hypoxic and consequently express more VEGF, which induces permeability. Thus indicating that VEGF also has heterogeneous expression pattern in the tumor.

Conclusion

VEGF was expressed by tumor, while Ang-1 and Ang-2 were induced in host infiltrating stroma cells in the presence of a tumor. Thus suggesting that vessels in different tumor regions will be exposed to different levels of VEGF, Ang-1 and Ang-2. This is expected to lead to a large heterogeneity in vascular maturation and permeability, as was detected here by MRI correlation maps and by fluorescence microscopy.

References: [1].Yancopoulos, G.D., *et al.*, Nature, 2000. **407**(6801): p. 242-8; [2]. Abramovitch, R., *et al.*, Cancer Res, 1999. **59**(19): p. 5012-6.; [3].Dafni, H., *et al.*, Cancer Res, 2002. **62**(22): p. 6731-9. [4]. Israely, T., *et al.*, Biol Reprod, 2003. **22**: p. 22.