A STUDY ON MICROVESSELS OF HUMAN BREAST CANCER TISSUES EMBEDDED IN EPOXY RESINS

Kukurtchyan, N. S.[a] and Karapetyan, G. R.[a]

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Tumor vasculature is irregularly sized and arranged in a disorganized manner, where they share characteristics of arterioles, capillaries, and venules simultaneously. Studies on microvessels of tissues (from mastopathy to human breast cancer) embedded in Epoxy resins using a light optical microscopy method were performed and human breast cancer microvessel images getting on semithin epoxy slices gave a new opportunity in the studying of this disease.

*Corresponding Authors

E-Mail: sapootraa_a@yahoo.com

[a] H. Buniatian Institute of Biochemistry, of National Academy of Sciences of Republic Armenia, Paruir Sevag str. 5/1, 0014, Yerevan, Republic of Armenia.

INTRODUCTION

The normal microvessels consist of arterioles, capillaries and venules, and form a well-organized, regulated and functional architecture,[1] but tumor vasculature is unorganized and has trifurcations and branches with uneven diameters. The vessel wall structure is also abnormal in tumors.[2] The formation of new blood vessels (angiogenesis) is required for the growth of most tumors. Angiogenesis in breast cancer helps fulfill the metabolic demands of the progressing tumor and plays a critical role in tumor metastasis.

Extensive laboratory data suggest that angiogenesis plays an essential role in breast cancer development, invasion, and metastasis.[3] Angiogenesis precedes the transformation of mammary hyperplasia to malignancy.[1,4] One of the most well-studied angiogenesis factors is called vascular endothelial-derived growth factor (VEGF). VEGF or other angiogenesis factors produced by tumor cells or nearby cells can cause the development of blood vessels that feed the growing tumor.[5,6] Hyperplastic murine breast papillomas and histologically normal lobules adjacent to cancerous breast tissue,[7-9] support angiogenesis in preclinical models, suggesting that angiogenesis precedes transformation of mammary hyperplasia to malignancy. Switch from sprouting angiogenesis to intussusceptive angiogenesis is an escape strategy from radiation-induced damage to the tumor vasculature.[10] Intussusception is another adaptive response of the tumor cells to stress and hypoxia. The transformation of one form of angiogenesis to another one will show the type of transformation of angiogenesis at human breast cancer.

However, it is still not clear how the alterations of large vessels could lead to such changes. This research aims to study microvessels of tissue (from mastopathy to human breast cancer) embedded in Epoxy resins using a light optical microscopy method.

MATERIALS AND METHODS

Reagents and chemicals: powdered paraformaldehyde, osmium tetraoxide, sodium cacodylate trihydrate; 96 % ethyl alcohol, acetone, Epon 812, Epon Hardener MNA, Epon Hardener DDSA, Epon accelerator DNP-30, Azur II and sodium borate were of analytical grade and purchased from Sigma Chemical Co. (USA).

In a current study at surgical procedures of primary operable human breast cancer**,** the biopsies of tissue located close to cancer tissue (3 patients control tissue), core biopsy (1 case of mastopathy), and core biopsy of breast cancer (10 patients) were taken. All procedures involved human subject were approved by the institutional review board/bioethical committee (Erevan State Medical University, RA) conformed to the Legal Aspects of Research Ethics and Science in European Community directive (2001/20/EC

Small pieces of tissue have immediately put in a cold mix of paraformaldehyde in a sodium cacodylate buffer and glutaraldehyde for 12 hours at 4 °C with following post-fixation in 1% OsO_4 solution for 2, then dehydration in ascending series of spirits; saturation in a mixture of acetone and Epon resins of different proportions to make gelatinous capsules were performed. Observation under a light microscope: semithin epoxy sections with up to 1 micrometer thickness were made using ultra cut Reichert (Austria) stained with Azur II (patent 2844 RA)[11] and studied under light microscope supplied with 40 x10 ocular lens.

RESULTS

As have shown the results of our study, using the current method of staining the material (human breast cancer tissue embedded in epoxy resins) by Azur II (patent 2844 RA) gives us very informative images of blood vessels for study.

At mastopathy, the blood vessels have insignificant lumen widening. Some capillaries have sprouted. The tendency of growing angiogenesis occurs.

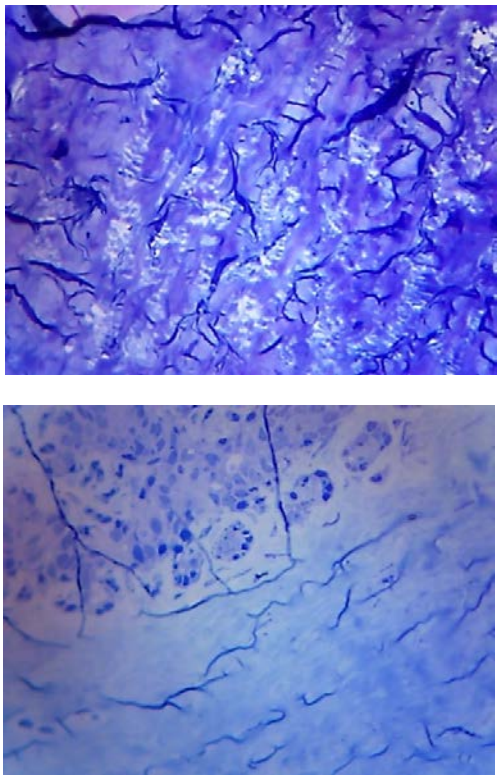

Figure 1. Capillaries at mastopathy

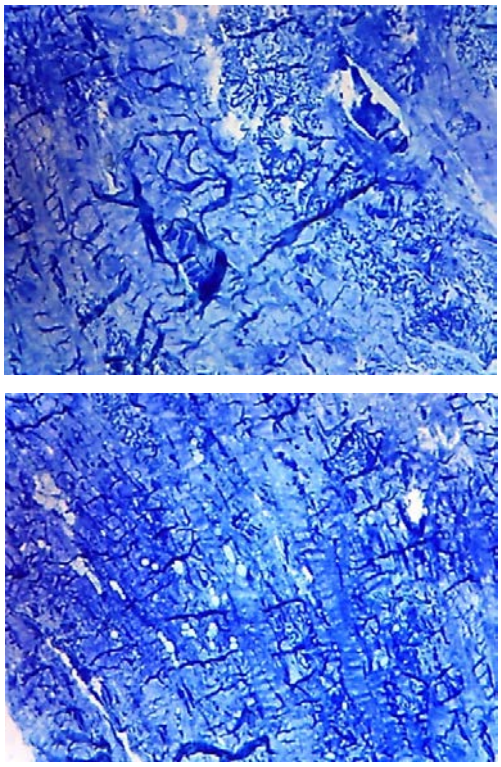

Figure 2. The control tissue of primary operable breast cancer

Blood vessels of breast tissue close to the primary operable tumor are large. Blood capillaries are presented with a high quantity with a different lumen wideness and shape. Many of them have sprouted.

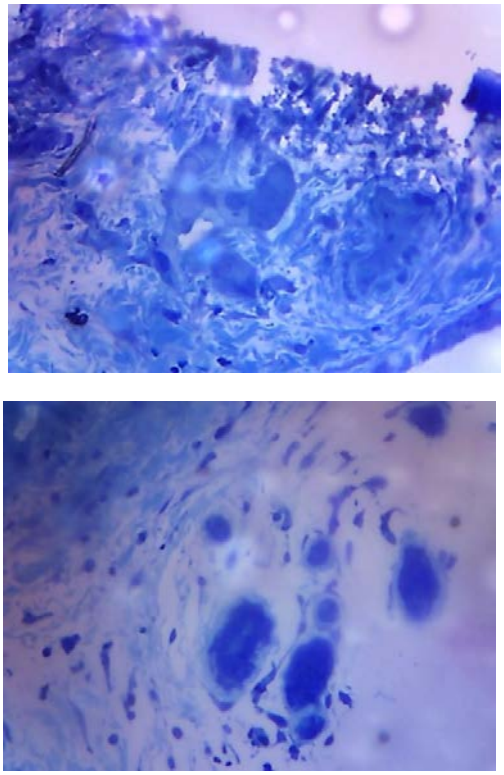

Figure 3. Human breast cancer x400

In studied cases at the II stage of cancer takes place the increasing of the quantity of large vessel profiles by intussusceptions (have one wall and different profiles shape).

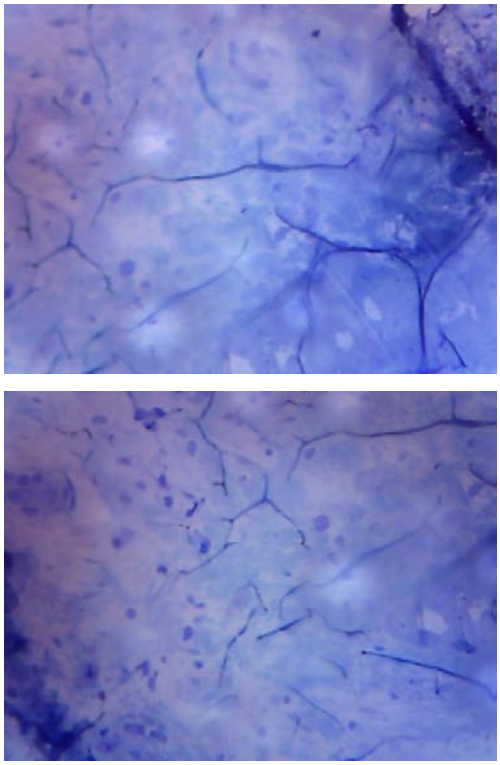

Figure 4. Stage II Human breast cancer tissue

Some of the capillaries with very narrow profile lumen increasing in quantity by intussusceptions. Areas of incompletely divided capillaries are observed.

DISCUSSION

Tumor vasculature is markedly distinct from normal vasculature in that blood vessel that supply tumor tissue are irregularly sized and arranged in a disorganized manner, where they share characteristics of arterioles, capillaries, and venules simultaneously, However, in tumor vasculature, sporadic blood flow is observed, leading to damaged capillary network systems.[12-14] At the same time, the mechanisms leading to it remain unknown.

Angiogenesis in breast cancer helps fulfill the metabolic demands of the progressing tumor and plays a critical role in tumor metastasis. Therefore, various imaging modalities have been used to characterize tumor angiogenesis.[15] Sprouting angiogenesis, the oldest model of microcirculation, is the de novo vessel formation from preexisting blood vessels. Splitting and hijacking, also known, respectively, as intussusception and cooption, are alternative models that account for tumor resistance to antiangiogenic therapy.[5] Despite exposure to antiangiogenic therapy and a subsequent significant decrease in microvascular density, mammary carcinoma had a rapid recovery post-treatment cessation, in virtue of intussusceptive pruning. Intussusceptive vessel growth involves bilateral centripetal protrusion of opposite endothelial cells lining the vessel wall.[16] Once in contact, the opposite vessel walls fuse and tiny apertures in the endothelial lining of the vessel walls form, ultimately leading to the splitting of the two newly formed vessels.[16,17] In contrast to sprouting angiogenesis, splitting angiogenesis is an energy-conserving mechanism as it does not dependent on a high rate of proliferation or basement membrane degradation and invasion, therefore saving energy and permitting the survival of the tumor despite hypoxia and stress.[16,18]

Tumor vasculature may be visualized using parametric imaging of specific morphological and physiological characteristics that collectively describe its properties.[19]

There is still uncertainty about angiogenesis as a prognosticator in breast cancer, with publications of conflicting results.[16] The tumor growth dependency on angiogenesis,[12,20] makes the hypothesis of angiogenesis as a prognosticator attractive.[21] Studies of the assessment of angiogenesis have mainly been based on this hot-spot approach, preferentially using the technique of counting microvessel profiles by all immunohistochemically stained distinct endothelial cells or cell clusters in a microscopic field.[22]

By the way dates of alteration takes place in large vessels are not taken into consideration at this very effective method, which becomes possible by staining of Azur 2 of semithin epoxy slices

In recent studies of tumor samples taken from 66 patients with T1-2 stages of invasive breast cancer of a non-specific type were stained with hematoxylin by Mayer and eosin, as well as by immunohistochemical method using antibodies to CD34 (the focus is on the evaluation of blood capillaries based on methodological facilities).[23] However, practice of tissue staining by Azur II on semithin epoxy slices does not ask using specific markers, sources, or involving additional methods for obtaining vessel images,[11] let us observe the vascular network of microvascular beds.

As have shown the results of this study, the alteration takes place in arterioles by the type of intussusception that can lead to damage to capillary network systems.

Beginning with the canonical sprouting angiogenesis vasculogenesis and intussusception, and finishing with vasculogenic mimicry, the need for different neovascularization mechanisms is further explored.[24]

CONCLUSION

Obtaining human breast cancer microvessels images on semithin epoxy slices gives new opportunities for further study of this disease.

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REFERENCES

[1]Jain, R. K., Molecular regulation of vessel maturation. *Nat. Med*., **2003**, *9(6)*, 685-93. DOI: 10.1038/nm0603-685

[2]McDonald, D. M., Choyke, P. L., Imaging of angiogenesis: from microscope to clinic, *Nat. Med*., **2003**, *9(6)*, 713-25. DOI:10.1038/nm0603-713.

[3]Ucuzian, A. A., Gassman, A. A., East, A. T., Greisler, H. P., Molecular Mediators of Angiogenesis. *J. Burn. Care Res*., **2010**, *31(1)*, 58. DOI:10.1097/BCR.0b013e3181c7ed82

[4]Bryan, P., Schneider, K., Miller, D., Angiogenesis of Breast Cancer. *J. Clin. Oncol.*, 2005, 23(8), 1782-1790. DOI: 10.1200/JCO.

[5]Salven, P., Ruotsalainen, T., Mattson, K., Joensuu, H. H., High pretreatment serum level of vascular endothelial growth factor (VEGF) is associated with poor outcome in small-cell lung cancer. *Int. J. Cancer*, **1998**, *79*, 144–146. DOI:10.1002/(sici)1097-0215(19980417)79:2<144::aid-ijc8>3.0.co;2-t

[6]Takahashi, Y., Kitadai, Y., Bucana, C. D., Cleary, K. R., Ellis, L. M., Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res*. 1995, 55, 3964–3968.

[7]Brem, S., S., Gullino, P. M., Medina, D., Angiogenesis: A marker for neoplastic transformation of mammary papillary hyperplasia. *Science*, **1995**, *880*, 1977-882,

[8]Carmeliet, P., Jain, R. K., Molecular mechanisms and clinical applications of angiogenesis. *Nature*, **2011**, *473(7347)*, 298-307. DOI: 10.1038/nature10144

[9]Djonoy, V., Andres, A. C., Ziemieski, A., Vascular remodeling during the normal and malignant life cycle of the mammary gland, *Microsc. Res. Tech*., **2001**, *52(2)*, 182-9. DOI: 10.1002/1097-0029(20010115)52:2<182::AID-JEMT1004>3.0.CO;2-M.

[10]Alameddine, R. S., Hamieh, L. and Shamseddine, A., From Sprouting Angiogenesis to Erythrocytes Generation by Cancer Stem Cells: Evolving Concepts in Tumor Microcirculation. *Shamseddine Biomed. Res. Int.*, **2014**, ID 986768. DOI:10.1155/2014/986768

[11]Kukurtchyan, N. S., Karapetyan, G. R., Patent 2844A, Rep. Armenia, **2014.**

[12]Bergers, G., Benjamin, L. E., Tumorigenesis and the angiogenic switch, *Nat. Rev. Cancer*, **2003**, *3*, 401–10. DOI:10.1038/nrc1093

[13]Foikman, J., *Tumor angiogenesis*, Mendelsohn, J., Howley, P. M., Israel, M. A., Liotta, L. A. Eds. *The Molecular Basis of Cancer*, **1995,** 206-232, W. B. Saunders Philadelphia,

[14]Fox, S. B., Tumour angiogenesis and prognosis, *Histopathology*, **1997**, *30, 294-301*. DOI:10.1046/j.1365-2559.1997.d01-606.x

[15]Hanahan, D., Folkman, J., Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*, **1996**, *86*, 353–64. DOI: 10.1016/S0092-8674(00)80108-7

[16]Djonov, V., Baum, O., Burri, P. H., Vascular remodeling by intussusceptive angiogenesis, *Cell Tissue Res.*, **2003**, *314(1)*, 107–117. DOI:10.1007/s00441-003-0784-3

[17]Burri, P. H., Hlushchuk, R., Djonov, V., Intussusceptive angiogenesis: its emergence, its characteristics, and its significance, *Dev. Dyn.*, **2004**, *231*, 474–88. DOI: 10.1002/dvdy.20184

[18]Ribatti, D., Nico, B., Floris, C., Mangieri, D., Piras, F., Ennas, M. G., Vacca, A., Sirigu, P., Microvascular density, vascular endothelial growth factor immunoreactivity in tumor cells, vessel diameter and intussusceptive microvascular growth in primary melanoma. *Oncol. Rep.*, **2005**, *14*, 81–84.

[19]Schatten, H., *Cell and Molecular Biology of Breast Cancer*, https://books.google.com/books?isbn=1627036342. **2013**, Springer Science-Business Media, New York, ISBN 978-1-62703-634-4

[20]Nishida, N., Yano, H., Nishida, T., Kamura, T. and Kojiro, M., Angiogenesis in Cancer, *Vasc. Health Risk Manag.*, **2006**, *2(3)*, 213–219.

[21]Siemann, D. W. The unique characteristics of tumor vasculature and preclinical evidence for its selective disruption by tumor-vascular disrupting agents. *Cancer Treat. Rev.*, **2011**, *37*, 63–74. DOI: 10.1016/j.ctrv.2010.05.001

[22]Hansen, S., Grabau, D. A., Sørensen, F. B., Bak, M., Vach, W. and Rose, C., The Prognostic Value of Angiogenesis by Chalkley Counting in a Confirmatory Study Design on 836 Brest Cancer Patients, *Mol. Oncol., Markers, Clin. Correlates*, **2000**, *6(1)*, 139-146

[23]Nikitenko, N. V., Senchukova, M. A., Tomchuk, O. N., Zaitsev, N. B., Stadnikov, A. A., Mitriako, S. O., Degtearev, A. M., Kirtbaia, D. V., Morphological Characteristic and Clinical Value of Different Types of Tumor Vessels in Breast Cancer, *Bioterap. Zh. Ross.*, **2016**, *15(3)*, 15-22. DOI: 10.17650/1726-9784-2016-15-3-15-22

[24]Zuazo-Gaztelu, I. and Casanovas, O., Unraveling the Role of Angiogenesis in Cancer Ecosystems, *Front. Oncol.*, **2018**, *8*, Article N. 248. DOI: 10.3389/fonc.2018.00248

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