LIGHT OPTICAL STUDY OF HUMAN BREAST CANCER TISSUE EMBEDDED IN EPOXY RESINS

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Keywords: human breast cancer; angiogenesis; light optical microscopy; epoxy resins.

A new possibility is shown for the study of the microcirculatory bed of human breast cancer tissue embedded in Epoxy resins. Using current method - staining the material by Azur II - it becomes possible the obtaining 3D images of blood vessels of human breast cancer tissue which gives new opportunities for further study of this disease.

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INTRODUCTION

Breast cancer is an increasing public health problem and usual combinations of local and systemic therapies are not always curative.[4] Blood vessels deliver oxygen and nutrients to every part of the body, but also nourish diseases such as cancer.[5,15]

It is well known that the most solid tumors has limited oxygen supply caused by lack of blood vessels as well as their destruction.[11] Extensive laboratory data suggest that angiogenesis plays an essential role in breast cancer development, invasion, and metastasis. Angiogenesis precedes transformation of mammary hyperplasia to malignancy.[3,10]

In spite the fact that capillary net around the tumor formed by normal endothelial cells, it is quite different from capillaries which have normal morphology, density and penetration.[21] The dense capillary net provides enough oxygen and necessary nutrients to developing tumor cells, as well as moves out the products of cells metabolism. The presence of capillary net leads to easier dissemination of metastatic tumor cells.[23]

So, for increasing the thickness of malignant tumor cells, the high specialized capillary net is required.

The formation of new blood vessels (angiogenesis) is essential for the growth of most tumors. 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.[1,11,18,20] Hyperplasic murine breast papillomas and histologically normal lobules adjacent to cancerous breast tissue[2,5,6] support angiogenesis in preclinical models, suggesting that angiogenesis precedes transformation of mammary hyperplasia to malignancy. Our current knowledge of tumor angiogenesis is based mainly on experiments performed in tumor-transplanted mice, and it has become evident that these models are not representative of human cancer.[14]

The aim of this study is to show the new possibilities for the study of the microcirculatory bed of human breast cancer tissue embedded in Epoxy resins.

MATERIALS AND METHODS

Reagents: Powdered paraformaldehyde; OsO_4; sodium cacodylate trihydrate; 96 % ethyl alcohol, acetone, Epon 812, Epon Hardener MNA, Epon Hardener DDSA, Epon accelerator DNP-30, Azur II, sodium borate. All reagent used were of analytical grade and purchased from Sigma Chemical Co. (USA).

The bioptate of tissue used in the current study were taken during Core biopsy (5 patients), as well as at surgical procedures before chemotherapy (5 patients).

All procedures involved human subject were approved by 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 h, then dehydration take place in ascending series of spirits; saturation in a mixture of acetone and Epoxy resins of different proportions to make gelatinous capsules were performed.

Observation under a light microscope: semithin epoxy sections with up to 1 μm thickness were made using ultracuts LKB (Swedish) and Reichert (Austria) stained with Azur II and studied under light microscope supplied with 40 x10 ocular lens.

RESULTS

The study of a 4-μm-thick section from each formalin-fixed and the paraffin-embedded tumor is generally accepted. Although the thickness of semithin epoxy slices is about 0.5 –1 μm, while the paraffin-embedded ones thicknesses are 3-4 μm. It means, hat the morphological results of tissues in semithin slices could be more informative than the paraffin embedded ones. Although the observation field of materials embedded in semithin epoxy slices under a light optical microscope is lesser than in case of the paraffin-embedded ones, however, the results are more informative.

As it can be seen from the results of our study, using Azure II staining method it becomes possible to obtain 3D images of blood vessels of human breast cancer tissue embedded in epoxy resins. So, we can say that this method is quite effective for the study of semithin slices of material taken at core biopsy as well as during surgical procedures (Fig.1, 2, 3, 4, 5) as it gives more opportunities for the study of human breast cancer by obtaining very informative images of blood vessels for their further study and analysis.

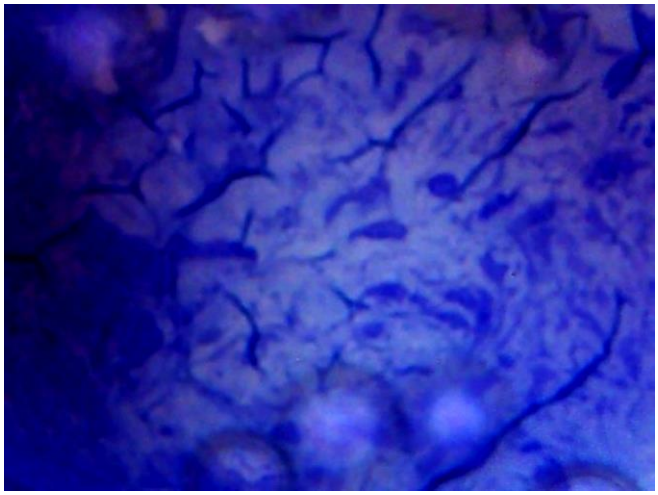

Figure 3. Blood vessels of human breast cancer tissue at core biopsy. 40 x10 ocular lens.

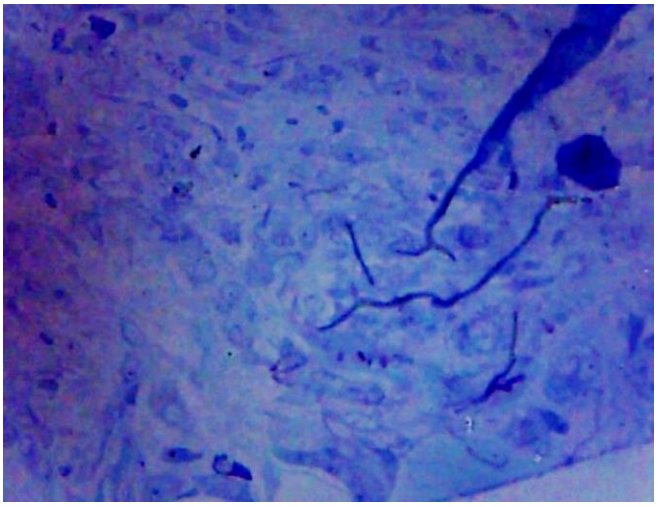

Figure 1. Blood vessels of human breast cancer tissue taken during surgical procedures. 40 x10 ocular lens.

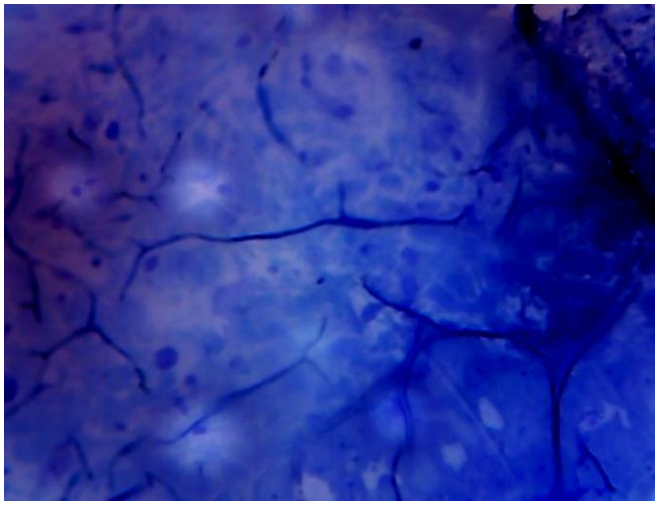

Figure 4. Blood vessels of human breast cancer tissue at core biopsy. 40 x10 ocular lens.

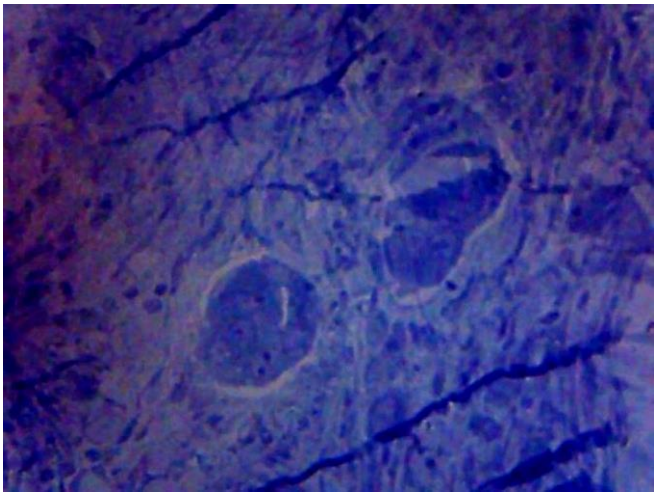

Figure 2. Blood vessels of human breast cancer tissue taken during surgical procedures. 40 x10 ocular lens.

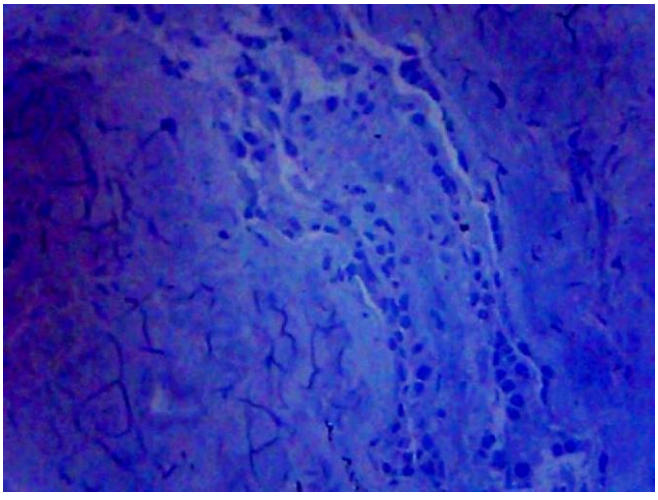

Figure 5. Blood vessels of human breast cancer tissue at core biopsy. 40 x10 ocular lens.

DISCUSSION

Recently, paraffin, celloidin and gelatin pouring of tissues have allowed to get histological specimens stained in different ways to carry out immunohistochemical investigations. However, the quality of tissue structures' preservation proved to be moderate.[9,17] The best preservation of tissue structures could be reached with epoxy injection of tissues.

The tumor growth dependency on angiogenesis[7,15] makes the hypothesis of angiogenesis as a prognosticator attractive, However, there is still uncertainty about angiogenesis as a prognosticator in breast cancer based on the publications contains conflicting results.[8] Studies of the assessment of angiogenesis have mainly been based on the hot-spot approach, preferentially using the technique of counting microvessel profiles by all immunohistochemically stained distinct endothelial cells or cell clusters microscopically. The method of tissue staining by Azur II do not request using any specific markers, sources, or involving additional methods for obtaining of images vessels.[13]

A useful method of angiogenesis estimation in human tumors is the microvessels counting method in the primary tumor using specific markers of endothelial cells such as VIII-factor, CD31, CD34, as well as the standard immunoperoxidase technique of vessels staining.[16,21,22] The studies on antibodies to CD31 factor was proved to be more sensitive method as the available panendothetial markers. This method recognizes more microvessels as other endothelial markers. CD34 and VIII-factor do not stain all vessels of the tumor, and antibodies to VIII-factor have reactions with lymphocytes as well. However, the specific characters of antibodies to CD31 are also not absolute, as they have a positive reaction with plasmatic cells. It must be mentioned that these markers stain normal, active/ proliferating endothelium. Since different studies used different techniques, it would be relevant to re-evaluate the prognostic value of angiogenesis by Chalkley counting in a confirmative study design.[19]

The importance of the Chalkley counting as an independent prognostic factor in breast cancer diagnosis, together with age, axillaries metastatic nodal involvement, tumor size, and histological grade of malignancy is high.[20] But it must be mentioned that Chalkley counting method, as well as other original methods, have limited abilities. Our method developed let us obtain the 3D images of vessels in human breast cancer tissue, which let us find the relation between angiogenesis types and molecular biological basic of human breast precancer types. The relations between transformations between each form of angiogenesis can show how could become the transformation of angiogenesis type to be a prognostic factor for disease, its early relapse and metastasis phase.

CONCLUSION

Obtaining blood vessels images of human breast cancer on semithin epoxy slices gives new opportunities for studies of tissues and recognition of this disease.

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Received: 01.05.2017
Accepted: 27.06.2017