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MACROPHAGE POLARIZATION MARKERS OF BLOOD MONOCYTES IN PATIENTS WITH BREAST CANCER

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Polarized monocytes can have diagnostic value in breast cancer, including a triple negative subtype. We examined the markers of M2- polarization of monocytes: CD163 and stabilin1 and the recently described chitinase-like proteins: SI-CLP, YLK39, YLK40, in breast cancer patients and healthy volunteers. The features of polarized monocytes in breast cancer were revealed depending on the molecular subtype of the tumor.

Keyworlds: monocytes polarization, chitinase-like proteins, inflammation, breast cancer.

Introduction

The appearance of polarized monocytes is noted in malignant neoplasms which are accompanied by inflammation in most cases. Such monocytes express marker molecules of macrophages exhibiting pro- (M1) or anti-inflammatory (M2) activity. A diagnostic value for CD163 + monocytes associated with M2-polarization was shown for breast cancer [1]. Recently, a number of studies have found new markers on tumor-associated macrophages that may be of clinical significance for the pathogenesis of breast cancer: the transmembrane protein stabilin1 and the associated stabilin interacting chitinase-like protein (SI-CLP), as well as the recently described chitinase-like proteins (CLP) YLK39 and YLK40 [2]. In the primary cultures of human macrophages Stabilin1 and CLP appear on M2-macrophages activated by the alternative pathway. CLP in blood monocytes are not known, but based on the results of studies of monocytes in vitro, they are markers of M2-polarization. Although not all producers of YLK39, YLK40 in the human body are established, a significant role in this process is assigned to tissue macrophages and blood monocytes.

For a triple negative breast cancer (TNBC), a special significance of the evaluation of the tumor microenvironment is shown [3]. There are few studies on circulating monocytes in TNBC. However, this subtype represents the most popular object for immunotherapy, which shows the relevance of the study of the effector mechanisms of the immune system in TNBC.

Thus, the goal was to evaluate the expression of markers of M2-polarization of monocytes in breast cancer taking into account the TNBC.

Subjects and Methods

Twenty-five patients diagnosed with primary local breast cancer and 6 healthy volunteers were included into the study. We examined monocyte populations and gene expression within them in peripheral blood of cancer patients before any treatment.

Phenotypic features of monocytes were assessed by flow cytometry using mAb (BD Pharmingen, USA) against CD45 and CD163 and kindly provided mAb Stabilin1 produced in Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, University of Heidelberg (Mannheim, Germany). Monocytes fraction from whole blood were subjected to positive CD14 Magnetic Cell Sorting using CD14 Magnetic beads (Miltenyi Biotech, Germany). Total RNA from the monocytes was isolated using RNAesy Mini plus kit (Qagen, USA). Real-time RT-PCR was performed with Taq-man technology on a Rotor-gene 6000 (Corbet, Australia). The Pfaffl method

was used to assess the target transcript in a patients group relative to that of an health donors control group using expression of an internal control GAPH-DH to normalize data.

Statistical analysis was performed using Statistica 10.0 software package. Differences between groups were evaluated using the non-parametric Mann-Witny U-test. The Spearman rank correlation coefficients were calculated by comparing the prevalence of monocytes subpopulations to the gene expression. The results are present as medians with interquartile ranges, Me (25-75%). Differences were considered significant where $p<0.05$.

Results

There were no differences in the content of M-2 polarized CD163 + monocytes in patients with breast cancer in comparison with healthy subjects: 1.55 (0.82-4.95) % and 2.05 (2.00-2.60)%, respectively. Stabilin1+ monocyte evaluation was performed only in women with breast cancer: it was -28.35 (13.20-58.30) %. However, the presence of the oncologic process affected the expression of the genes of CLP YLK40, YLK39 and SI-CLP, which was significantly decreased in comparison with the group of healthy individuals, amounting to 0.64, 0.7, and 0.54 with respect to healthy controls, respectively. Correlation analysis showed that the expression of SI-CLP ($r = -0.8$, $p> 0.05$), YLK39 ($r = 0.8$, $p> 0.05$) and YLK40 ($r = 0.4$, $p>0.05$) was not associated with the number of M2-polarized CD163 + monocytes in BC. This fact reveals a difference in the mechanisms that induce the synthesis of CLP and the CD163 molecule in monocytes.

It is known that TNBC is characterized by a more pronounced reactivity of the immune system compared to other molecular subtypes [3, 4]. The results obtained in our study also confirmed this thesis. Thus, the content of M2-polarized CD163 + monocytes was lower for TNBC (5.70 (3.30-9.90) %) compared with other molecular genetic subtypes (1.45 (0.65-4.10) %) (Figure 1). The content of Stabilin1 + monocytes in TNBC (9.60 (0.10-19.10) %) was higher for a triply negative subtype - 43.35 (25.85-66.85) % (Figure 1), however, differences in gene expression CLP (the putative markers of M2-polarization of monocytes), as a function of the molecular subtype, were not detected (Figure 1).

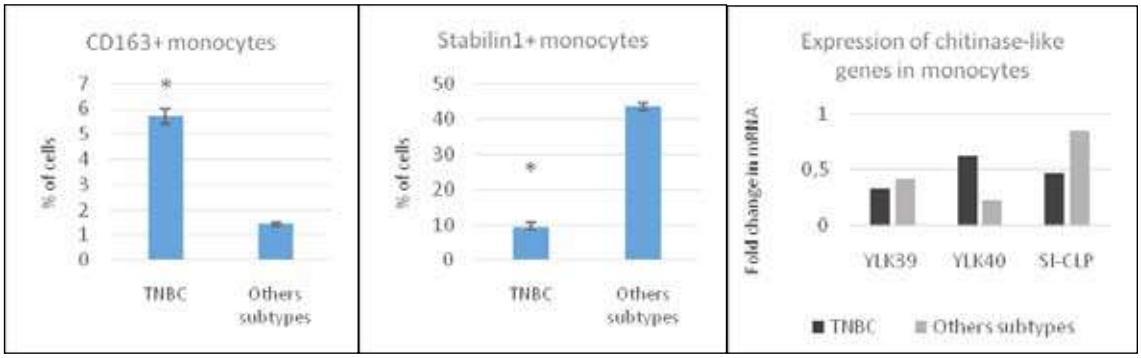

Figure 1. Features of the PB monocytes in patients with TNBC (p-level: * - p<0,05).

Conclusions

In general, the diagnostic significance of CD163+ and Stabilin1+ mnocytes has not been established for breast cancer. However, their significance for the characterization of TNBC is shown. Data were obtained on the presence of CLP in monocytes of both healthy people and patients regardless of the molecular subtype of the tumor, which may indicate the polarization of monocytes at the level of transcription of CLP genes. At the same time, the tumor process can have a negative effect on the expression of these genes in monocytes, reducing it. The obtained data reveal the significance of the study of CLP as markers of monocyte polarization. Identified features of TNBC indicate new diagnostic parameters for this subtype.

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SIGNAL TRANSDUCTION AND THERAPEUTIC EFFECTS OF HUMAN MENINGIOMA EMBOLIZATION

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Hypoxia plays an important role in the pathogenesis of meningiomas, and hypoxia can stimulate apoptosis of tumor cells; on the other hand, hypoxia may contribute to meningioma progression. To clarify the molecular basis for the differences in the outcomes of these two therapeutic strategies, we investigated the mRNA expression of *HIF-1α*, *AhR*, *ARNT*, *NcoA2*, and of HIF-1α-controlled genes *VEGF-A*, *GLUT1*, and *c-Myc* in tumor samples of *benign meningiomas* from two groups of patients: where the tumor was removed either without or after endovascular embolization. The study showed that in *meningiomas* exposed to hypoxia as a result of embolization, the mRNA levels of *AhR*, *ARNT*, and *NcoA2* were significantly lower as compared to unembolized meningiomas. It is likely that AhR can be an independent therapeutic target in meningiomas without the intermediation of endovascular-embolization–induced hypoxia.

Keywords: meningiomas, AhR, HIF-1α, hypoxia, embolization, therapeutic target.

Relevance

Hypoxia plays an important role in the mechanisms of development of meningiomas, and hypoxia can stimulate apoptosis of tumor cells; on the other hand, hypoxia can contribute to meningioma progression. The central role in the development of hypoxia is played by the transcription factor HIF-1α and its downstream signaling pathways mediating angiogenesis, glucose metabolism, and cell proliferation. In the nucleus, HIF-1α forms a complex with the ARNT protein [*arylhydrocarbon receptor (*AhR) *nuclear* transporter], and *AhR can compete* with HIF-1α for ARNT. *AhR is known* to control the expression of genes of cytochrome P450 family 1 and to participate in the molecular cascades that promote cell differentiation and apoptosis and inhibit proliferation. It is known that the transcription coactivator NcoA2 regulates activation and inhibition of proteins of the basic helix-loop-helix (*bHLH*)/*PAS family,* which includes HIF-1α, ARNT, and AhR. The aim of the study was to investigate HIF-1α- and AhR-dependent signaling pathways in unembolized and embolized human meningiomas to reveal the molecular basis of a tumor response to hypoxia. This knowledge can be relevant to the search for molecular markers of different responses to hypoxia and ultimately the choice of therapeutic tactics.

Material and Methods

The object of the study was tissue samples of *meningiomas*, a type of *benign* brain tumor, from two groups of patients with the diagnosis of Meningioma WHOGr1, where the tumor was removed either without (n = 10) or after (n = 7) endovascular embolization. The mRNA expression of *HIF-1α*, *AhR*, *ARNT*, and *NcoA2* and of HIF-1α-controlled genes *VEGF-A*, *GLUT1*, and *c-Myc* was determined in the samples. The gene expression was evaluated on the iCycler CFX96 real-time PCR detection system (Bio-Rad Laboratories, USA) according to the TaqMan principle. *EIF2B1* served as an internal control (housekeeping gene). The $2^{-\Delta Ct}$ method was used to analyze the data. All the calculations were performed in the Statistica software package (StatSoft, Inc., USA). Data were subjected to analysis of variance (ANOVA) with the *Bonferroni post hoc* test.

Results

The study showed that there were no statistically significant differences in the mRNA levels of *HIF-1a*, *VEGF-A*, *GLUT1*, and *c-Myc* between the unembolized and embolized meningiomas. Nevertheless, in the *meningiomas* exposed to hypoxia as a result of embolization, the mRNA levels of *AhR*, *ARNT*, and *NcoA2* were significantly lower as compared to the unembolized meningiomas.

Conclusions

Thus, in the mechanism of action of hypoxia on meningiomas (as a result of endovascular embolization), the AhR-dependent pathway plays a more important role than does the HIF-1α-dependent