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Research Article

Tissues Protein Microenvironment and Survival by Age at Cancers

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Abstract

Introduction: Three general types of survival by age characterize the effectiveness of conventional cancer treatment in a population of developed country. To explain big difference between received results, the microenvironment of normal tissues investigated and compared with clinical data.

Material and Method: Open database for cancer survival by age in UK and open database for human proteins in normal human tissues used for extraction of original information for 16 cancers and 18 proteins in normal tissues, which are host/primary for corresponding malignancy. All original data for protein levels transformed by normalization to level in bone marrow. The transformed data were analyzed and compared with three general types survival by age, using statistical instruments of Excel.

Results: Three cohorts of sixteen cancers of UK population's data differ around 7 times according to exponential rate of survival's decline by age. The average result of therapy in cohorts is better, the higher the average level of markers for hematopoietic (CD34) and vasculogenic (CD31) cells in normal tissues corresponded to cancer's cohorts. The level of immune markers for T-, B-cells, and neutrophils in normal tissues are lower, than stem cells, young lymphoid descendants, and endothelial ones. The result of therapy in cohorts is the better, the lover level of immune markers in normal tissues, corresponded to cohorts i.e. the more shift to the "stemness" in the differentiation's range. The number of markers for hematopoietic stem cells and their descendants in normal tissues varies in the opposite way.

Discussion: Despite common domination of hematopoietic stem cells markers in normal tissues, their number may vary by physiology of inter-organs flows of the fluids. The higher morphogenic resource of CD34 and CD31 in normal tissue can extend the local tumor growth, delay a coming hypoxia, metastases, and death.

Conclusion: The proteins microenvironment of normal human tissues, as a host of unpredictable malignancy, does not correspond completely to the usual markers in terms of circulating blood cells. The domination of "CD34- stemness" and "CD31-vasculogenity" provide a local longevity of tumor and thus delay the shift of its logarithmic growth into quasi-linear, the offensive of hypoxia, followed metastases and death.

Keywords: Survival; Age Population; Cancers; Normal Host-Tissues; Protein Tissue Markers; Hematopoietic Stem Cells; Circulating Cells

Introduction

Earlier, we considered a five-year survival by age at diagnosis for 20 invasive cancers in the US population and argued the aggregating them into 3 really different cohorts of tissues, as a favorite, intermediate, and unfavorable for 5 years survival by age [1]. The cohorts are not explainable by the turnover of cells in

original normal tissues and their affiliation to germ layers (ecto-, meso-, or endoderm), or clusters of gene expression in them, estimated in [2]. There is no base to substantiate and validate cohorts by blood cells characters. The dependence of cancer activity on the microenvironment of host tissue/organ is a part of the entire problem. Publishing of the normal tissue's protein data [3] opens opportunities for feather analysis of the reasons for 3 general types of cancer survival. The aim of the present study is 1) to validate 3 types of cancer survival rates by age in the other, UK population, and 2) to investigate their dependence from tissue's proteins of normal organs, which are original for cancers each of types.

Materials and Methods

Survival for cancer in different target organs

Data about five-year net survival by age at cancer diagnosis for England population 2009-2013 have extracted from an open database at last reduction 11 May 2016 [4,5]. According to previous conclusion [1], both male and female cancer cases divided on three cohorts: A (liver, lung, esophagus, pancreas, and stomach), B (cervix uteri, kidney, larynx, bowel, and ovary), and C (uterus, thyroid, bladder, prostate, testis, and breast). Each of A, B, and C cohorts divided by age at diagnosis on seven subgroups: average age 27 (15-39), 44,5 (40-49), 54,5 (50-59), 64,5 (60-69), 74,5 (70-79), and 89,5 (80-99).

The exponential approximation used for three (A, B, C) 5 years-survival curves by age generated for seven age-subgroups on semi-logarithmic plots [6]. Two single exponents characterized each one of A, B, and C survival curves, as they have biphasic patterns. Two linear regions on each curve fitted by exponential functions for that using the simplest equation:

$$S = e^{-kt}$$

Where S was survival in relative units, t was the elapsed time in years, and k was the exponential death rate per year.

The proteins in normal tissues, which are potential target for cancer

The level of proteins insight normal human tissue/organ extracted from [3] used for interpretation of a cancer survival's specificities in tissues A, B, and C types. Along with normal proteins in esophagus, liver, lung, pancreas, stomach (A), bowel, cervix uteri, kidney, larynx, ovary (B), bladder, breast, prostate, testis, thyroid, uterus (C), the proteins of bone marrow used as a referent also. The fifteen proteins for each of organ included: CD1c, CD3d, CD2, CD7, CD19, CD25, CD25FOXP3, CD27, CD34, CD31, CD33, CD105, CD133, PD-1L (CD274), PD-1(CD279), Ki-67, DNA polymerase μ (POLM), and template-independent deoxynucleotidyl transferase terminal-interacting protein 1 (DNTTIP1), which enhances activity of template-independent DNA polymerase DNTT (TdT) catalyzed the random addition of deoxynucleoside 5'- triphosphate to the 3'-end of a DNA initiator (primer). Most of them show the cells originated of bone marrow, excluding CD105 associated with the endothelial stemness in all tissues of the body. All detectable levels of transcribed mRNA molecules (nTPM) for each of fifteen proteins were normalized preliminary to the corresponding nTPM in bone marrow. A using such relative protein's level (RPL) let us a correct comparison of tabulated in [3] data in terms "protein vs. protein" and "tissues vs. tissues".

Statistical analyses

The data compared by mean value (M), standard deviation (SD), standard error (SE), t-criterion and probability p. The trends of the aggregate parameters evaluated by mathematical functions generated automatically in the Excel program. The coefficient of determination R^2 used as a statistical measure of the goodness of fit of the regression line to the data. R^2 confirmed by Equation (2) for its t -parameter:

$$t = R^2 \times (n-2) \times (1-R^2).$$

Results and Discussion

According to figure 1, the 5-years survival reduces exponentially by 3.9-2.9%, 0.8 -1.4%, and 0.2 - 0.8% per year in cancers cohorts, separating corresponded A, B, and C' normal tissues types.

Figure 1: Population 5-years survival by age at diagnosis in the cohorts of cancer A, B, and C.

Abscissa: age at diagnosis, years. Ordinate: net 5-years survival, percentage. Circles- M ± SD. In the boxes: the formulas of an exponets approximated of biphasic survival curves two single exponents for each of curve.

Formally, this declining of average k-values values (y) by A to C fits by linear approximation y = -0.0145x + 0.0457 (R^2 = 0.8218; p = 0.014) or logarithmic approximation y = -0.027ln(x) + 0.0329 (R^2 = 0.8878; p = 0.005). Thus, the diversity of survival curves to three main types is possible, as we proposed earlier [1]. They are unfavorable (A) with averaged kexp = 0.034, intermediate (B) with k_{exp} = 0.011, and favorable (C), with k_{exp} = 0.005 per year. This decreasing of exponential death rate k_{exp} does not correlate with RPL of some proteins in normal tissues A, B, and C (Figure 2).

Figure 2: Changes of normal RPL, that do not fit the changes of A, B, C - cancer survivals in figure 1**.**

Abscissa: upper text indicates the proteins types (i), lower letters show unfavorable (A), intermediate (B), and favorable (C) normal tissues. Ordinate: average RPL of i-proteins The lines and formulas in boxes describe the binomial approximation of (nTPMi/ nTPMbm*) changes from A to C. *nTPMbm detectable level of transcribed molecules mRNA in bone marrow for CD105, CD133, PD1, CD25Foxp3, Ki67 (19, 4, 0.9, 1.3, 46.7). POLM protein involves in repair of DNA double-strand breaks, and DNTTIP1 protein enhances of TdT- activity (both. are not shown in figure2).

Convex and concave binomial approximations of proteins changes from A to C cohorts do not suppose any involvement of CD105, CD133, CD25FOXP3, PD-1, and Ki 67 proteins in unidirectional declining of cancers' resistance to the therapy from A to C. Additional analysis confirmed (p = 0.045) a lower level of DNTTIP1 RPL in unfavorable tissues A (0.46 ± 0.04) in comparing with average RPL for jointed B+C tissues (0.57 ± 0.04). It does not contradict to lower level of CD34 in A, because about 15% of the CD34+ cells are DNTT (TdT)-positive lymphoid progenitors [7].

The figures 3 and 4 show the proteins, which correlate completely with changes of cancer survival in a range of A→C types.

Figure 3: Changes of normal RPL, that fit the trend of A, B, C - cancer survivals in figure 1.

Abscissa: upper text indicates the proteins types (i), lower letters show unfavorable (A), intermediate (B), and favorable (C) normal tissues. Ordinate: average RPL of i-proteins. Vertical boxes show SE for each of white columns in A, B, and C normal tissues. The lines and formulas in horizontal boxes describe the linear approximation of (nTPMi/ nTPMbm*) changes from A to C. *nTPMbm detectable level of transcribed molecules mRNA in bone marrow for CD2, CD27, CD33, CD3d, CD19 are 11.5, 10.7, 32.7, 28.8, 6.3.

According to figure 3, the level of CD2 T cells' protein in normal tissues of unfavorable type A exceeds such in bone marrow, whereas in favorable tissues of C type, it is 3-times lower. The level of CD3d T cells' protein has the same trend (gray columns; Figure 3). The levels of B cells' protein CD27, CD19 (gray), and neutrophils CD33 decline monotonously from A to C types as well. It is strange, that unfavorable tumors A emerge in normal tissues with the highest content of T cells signs, and vice versa for favorable one C. A ratio neutrophil to lymphocytes proteins (NLR) in all tissues A, B, C is much lower (M ± SE = 0.098 ± 0.0125; Figure 3.) than it is in normal blood (around 2-3) [8]. Thus lymphocyte' proteins may penetrate in tissues selectively, and this penetration does not seem to relate to any immune protection.

The CD7, CD5, CD25, CD31 proteins are markers of consecutive stages of maturation of T-cells from CD34 HSC to differentiated CD2, CD3 cells (Figure 4). They decrease by 1.7, 3.6, 3.5, and 2.8-times, including ligand PD-1L (3.5 times). A descendant CD25, CD5, PD-1L and CD7 co-expression with CD34 stem cells and TdT "prolymphocytes" also, varying from 5 to 30 percentage [9,10].

Figure 4: Proteins content in normal organs, which correspond to those that were original for cancer in cohorts A, B, and C in figure 1.

Abscissa: upper text indicates the proteins types (i), lower letters show unfavorable (A), intermediate (B), and favorable (C) normal tissues. Ordinate: average RPL of i-proteins The lines describe the approximation of (nTPMi/ nTPMbm*) changes from A to C with $R^2 = 1.0$ ($p < 0{,}001$) for all of them. *nTPMbm detectable level of transcribed mRNA molecules in bone marrow for CD34, CD25, CD5, PD-1L, CD31, CD7 are 5.8, 0.4, 12,4, 2.5, 35.8, 28,8.

Thus, parallel decreasing of markers for pre-thymic T-lymphoid progenitor-prolymphocytes CD7, primitive intrathymic T-lymphoid precursors CD5 [9,11,12], and CD25 associated with "regulatory" lymphocytes-Treg [13] point on weakening of stem cells differentiation along with A, B, C tissues. The common level of unmatured cells exceeds the level of matured CD2 and CD3d T cells in figure 3, indicating a quantitative prevalence of young γδ lymphocytes in all tissues A, B, and C also [14,15].

A decrease of ligand PD-L1 level from A to C tissues can lied to the weakening of inhibition of programmed cell death protein (PD-1), debated as a cause of enhance of immune evasion of tumors [16]. However, PD1 level in A, B, and C normal tissues in figure 2 oppose to this mechanism, ranging inappropriately. A weakening inhibition of CD34 hemopoietic stem cell is more probable, as they express PD1 too [17]. This expected mechanism is in accordance with linear arising the CD34 level by 5.5, 8.6, and 11.2 ($R^2 = 1.0$) in A, B, and C tissues (Figure 4). Thus, the lower level of PD-1L, the higher level of CD34, and vice versa. The maximal saturation by stem cells' marker in C tissues is most favorable for cancer prognosis.

Along with a dominant increasing of CD34 in tissues (5.46, A; 8.64; B, and 11.24; C), the endothelial markers CD31 protein, researchers identified 'vascular progenitor', increases also from 0.6, to 1.04, and 1.41 ($R^2 = 1.0$) (Figure 4). Only some of a CD34 bone marrow' emigrant can be on early stage of CD31 angiogenic differentiation/commitment. However, an immune-histochemistry analysis of human aorta shows the prevalence of CD34 on CD31 markers [18]. The percentage of CD31 toward CD34 is unchangeable for all tissues A, B, and C with min-max = 10.9% -12.5% and M ± SD = 11.8 ± 0.44%. This supposes the probable double- positivity of both proteins. A level of CD34 stem cells co-expressed the CD31 in bone marrow varies wide from 0% to 60% [19]. Oppositely, the presence of CD34 marker in CD31 blood' cells is about 2% [20], or even 0.12-0.29% [21]. Therefore, the relative increase of CD31 level in A → C tissues (Figure 4) is most likely a result of penetration of double positive CD34+ cells from bone

marrow. Otherwise, the only preferential transition of circulating double-positive CD34 CD31 cells from blood into tissue cannot exclude.

Therefore, we confirmed for UK population the three types of cancers survival by age. Unfavorable A, intermediate B, and favorable C types relate to different normal tissues, as it discussed earlier for USA [1]. The simplest reason for that seemed the dependence of cancer's aggressiveness from blood flow distribution in different tissues [22]. According [22] average 40% of the variation in the metastatic distribution can be attributed to blood circulation. Using the data of this article, we evaluated average cells cross-flows in seven organs, which became 1.65 ± 0.64 for lung, liver, pancreas, and stomach (A), 1,2 ± 0 for bowel and kidney (B), and zero for prostate (C). These M ± SD show the high blood flow's interfering of six other tissues with average A-tissue and lack of such interfering with C-tissue. So, We might have suggested, that the more flow interfering in an organ, the less probability of HSC's homing in it (Figure 4).

Most of used nTPM are markers for circulating hematopoietic cells.

We found, that CD34 cell dominate in A, B, and C tissues (5.5, 8.6, and 11.2) over total T cells CD2 (1.13, 0.76, and 0.37), meanwhile their ratios in bone marrow and blood quite opposite [3,23].

An A, B, C normal tissues specifies by stable content (11.8 ± 0.44%) of CD31 cells among CD34 cells.

And, the worse cancer survival the lower CD34, CD31, DNTTIP1, and higher CD2, CD25, CD7, CD5, PD-1L in normal tissues, and vice versa.

Though common function of CD34 proteins has not explained yet, understanding the links between cells suggests some role of CD34 HSC in development and renewal of many tissue types. HSC may transfer the protein CD34 in many tissues with cells like epithelial stem cells, endothelial progenitors, enterocytes, keratocytes, interstitial cells, fibrocytes, muscle satellite cells, salivary gland stem cells, and on, where the CD34 had found [24]. The transdifferentiation/commutation/commitment of stem cells might be one way of such transmittance. For an example, double-positivity with protein CD31 switch the potency of CD34 MSC to angiogenesis [19,20]. In this view, probable higher level of double positive CD34 CD31 endothelial cells in normal C-tissue can relate to their advantages for an avoiding of cancer mortality, in comparing with A-tissues. Such an opportunity bases on the unchangeable percentage of CD31 toward CD34 and strong linear dependence of CD31(y) on CD34 stem cells level (x) in A, B, C tissues: y = 0,14x - 0,166; R^2 = 1.0, p < 0,001.

The increase of CD31(y) by decrease of CD2(x) along with A, B, C: y = -1,06x+1,82; R^2 = 0.996, p = 0,045 may point on naïve nature of some CD31 T cells. The loss of these cells in human blood by 1-7% per year of age accompanies by loos of proliferation capacity 1-1.3% per year and telomere length on 20-24%, [25]. The shortened telomeres of naive T cells after accelerated proliferation comes at a deadly cost to the aging host. Thus, the lack of CD 31 naïve T cells in normal A-tissue can be the real cause of unfavorable "aging" immune microenvironment as well, as the lack of CD34 stem cells. High presence of highly differentiated CD2 in A -tissues (20,5%) is better interpreted as unfavorable, than favorable event, because the recent thymic emigrants dominate in young humans event, but an elderly have a prevalence of cells with replicative senescence [26]. Tissues A are less viable in comparing with tissues C. Thus, CD34 and CD31 seem a providers of somatic stability of

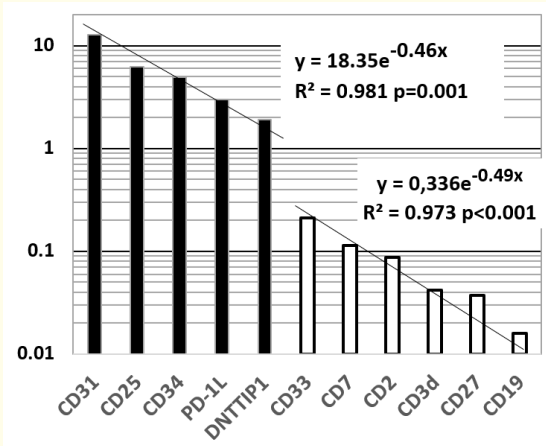

Figure 5: RPL of different proteins in placenta. Abscissa: the proteins that correspond to Ordinate: RPL (TPMpl/TPMbm) of different proteins in normal placenta. Black columns: M ± SD = 5.79 ± 1.92. White columns:0.11 ± 0.036; p = 0,02.

normal tissues,which are potential target for cancers. In contrast to this, lymphoid descendant of stem cells, namely naive CD5,CD7 and Treg CD25 relate to microenvironment, which favorite for malignant tissues. Nevertheless, all of them are responsible for tissues growth.

To check the matter of terms" favorable/unfavorable", the RPL for placenta is considered as an example of adaptor/controller of growing tissue (Figure 5).

In term of cells, CD34 HSC, committed mostly to an angiogenesis by CD 31 protein are the main promoter of tissue growth process [27,28]. High levels of CD25 and PD-1L are rather signs of young lymphocytes, because they relate to lymphopoiesis in thymus [29,30] and express on CD34 HSC [10,31]. DNTTIP1protein take part in enhancing the angiogenic potential of CD34+ as well [7,32]. The equations below show the exponential dependence of survival decline's rate (y, Figure 1) on CD34 RPL and CD31RPL (x, Figure 4):

$y = 0{,}2522e^{-0{,}368x}$; $R^2 = 0{,}9159$; $p_{CD34} = 0{,}003$-------- 1)

$y = 0{,}1628e^{-2{,}629x}$; $R^2 = 0{,}9157$; $p_{CD31} = 0{,}003$ ---------- 2)

Thus, cells a favorable microenvironment in tissues provides by high level of stem cells and young lymphoid descendant cells. The question is how the level of trophic cells may control the survival's rate. A recruitment of endothelial progenitor cells CD34CD31 from bone marrow follows by vessel formation in malignant tissues as well [33]. The lower level of CD34 and CD31 in unfavorable tissues A means hardly a deficiency for providing of vessels formation, because their original reserve in normal tissues A exceeds more the 5 times that one in bone morrow (Figure 4). However, in comparing with reserve of favorable tissues C, the reserve in "A" is limited. Correspondently, slowing-down of tumor's growth from exponential to quasi-linear [34], hypoxia, metastases and death [35,36] come earlier as well. That is what the Figure1 shows. For instance, the average rate of loss of a 5-years survival by age declines six times from A to C cancers. Importantly, that most cancers at the time investigation in clinic, are soon hypoxic as far as a quarter of physoxia (normoxia). The physoxia is roughly 5,3% of oxygen in lung, liver, brain (A), 6,3% in cervix, kidney, and rectum (B), and 8,5% in a breast (C) [36], i.e., correlate with the levels of CD34 and CD31 in figure 4.

Conclusion

Three cohorts of sixteen cancers of UK population's data differ around 7 times according to exponential rate of survival's decline by age. The average result of therapy in cohorts is better, the higher the average level of markers for hematopoietic (CD34) and vasculogenic (CD31) cells in normal tissues corresponded to cancer's cohorts. The level of immune markers for T-, B-cells, and neutrophils in normal tissues are lower, than stem lymphoid descendants, and endothelium cells. The result of therapy in cohorts is the better, the lover level of immune markers in normal tissues, corresponded to cohorts. Despite common domination of hematopoietic stem cells markers in normal tissues, their number may vary by physiology of inter-organs flows of the fluids. The higher morphogenic resource of young lymphoid descendant, CD34 and CD31 in normal tissue can extend the local tumor growth, delay a coming hypoxia, metastases, and death.

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Conflict of Interest

None of conflict of interest exists.

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