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Expression of Pu.1, C/Ebpα and Bach1 Transcription Factors in Immune Cells in Patients with Cancer

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Abstract

Dysfunction and abnormal differentiation of immune cells and hematopoietic precursors are well described in patients with cancer, although the role of transcription factors in these defects is not well established. Here, we evaluated expression of C/EBPα, PU.1 and BACH1 transcription factors in lymphoid and myeloid cells, including NK cells, T cells, neutrophils and monocytes, isolated from healthy donors and cancer patients. The results revealed a significant suppression of transcription factors participating in the development of immune cells in patients with cancer. Analysis of expression of transcription factors in $CD34^{+}CD45^{dim}$ hematopoietic precursors showed that in contrast to the downregulation of transcription factor in mature peripheral blood immune cells, expression of these regulators in stem/precursor cells was significantly increased in patients with lung cancer, especially in patients with detectable metastases. These results open a new opportunity to test whether altered transcriptional regulation of mature immune cells in cancer may be intrinsically related to abnormal functioning of transcription factors in early hematopoiesis or occurs independently via direct effects of tumor-derived factors on circulating immune cells.

Keywords: NK cells, T cells, neutrophils, monocytes, C/EBPα, BACH1, PU.1

Introduction

Cancer remains one of the leading causes of death worldwide despite a significant progress in cancer diagnostics, treatment and prophylactics during last decades. It is generally accepted that the development and progression of cancer are closely associated with dysregulated functioning and diminishing of local and systemic immune responsiveness. Immune abnormalities in cancer may be due to the effect of tumor/stroma-derived factors on mature circulating immune cells or on their precursors in the bone marrow. The hypothesis of intrinsic transcriptional defects in the differentiation mechanism of hematopoietic stem and progenitor cells as a source of dysfunction of immature and mature immune cells in cancer patients is supported by a growing number of recent findings. A significant reduction in c-myc expression was observed in both NK cells and T lymphocytes in patients with cancer [1,2]. There is evidence of a high level of c-myc expression in neutrophils in cancer [3]. Cancer-associated abnormalities in differentiation of hematopoietic progenitor cells and in myeloid and lymphoid cells have also been reported [4-7]. Thus, it is conceivable to speculate that an abnormal expression of proto-oncogenes

in lymphoid cells and myeloid cells may be due to a general imbalance of transcription factors responsible for the lineage-specific differentiation.

Leukocytes' fate is progressively determined during the gradual differentiation of hematopoietic stem cells (HSCs) into multipotent progenitors (MPPs) and common lymphoid or myeloid progenitors [8]. Transcription factors are key regulators of these cells acting through their capacity to bind DNA and control gene transcription, which thus regulates the development of specific hematopoietic lineages from stem cells. Among transcription factors C/EBPα/β, BACH1/2 and ETS play pivotal roles in myeloid and lymphoid cell differentiation. However, the gradual mechanisms by which differentiation of common progenitors controlled under cancerous condition have been unclear.

C/EBPA (CCAAT/enhancer binding protein (C/EBP) alpha), a protein encoded by the *C/EBPA* gene in humans, was originally identified 30 years ago as a transcription factor that binds both promoter and enhancer regions. Analysis of its function in hematopoietic malignancies as well as in solid cancer has revealed many ways how cancerous cells abrogate C/EBPα function [9-11]. Although early studies focused on the role of C/EBPα in regulating transcriptional and differentiation processes during liver and adipogenic development, recent reports provide more mechanistic insights into its role in normal and malignant hematopoiesis [12,13]. For instance, C/EBPα has been reported to protect adult HSCs from apoptosis and to maintain their quiescent state. Consequently, deletion of *C/EBPA* is associated with loss of self-renewal and HSC exhaustion [14] as well as the absence of mature neutrophils and eosinophils in mutant mice [15]. Thus, C/EBPα functions as a priming factor at the HSC level where it supports myeloid differentiation and reduces lymphoid lineage selection, which forms the epigenetic shape of HSCs for balancing various cell fate choices.

Analysis of C/EBPα target genes essential for myeloid cell development identified *c-myc* as a C/EBPα negatively regulated gene in myeloid cell lines [16]. The fact that C/EBPα directly affects the level of c-myc expression and, thus, the granulocytic differentiation pathway, is intriguing as it has been previously shown that down-regulation of c-myc can induce myeloid differentiation [17]. As proliferation and differentiation are mutually exclusive, c-myc - a proliferative factor, and C/EBPα - a differentiation factor, work in opposition to each other. Given that c-myc expression is down-regulated in NK and T cells in patients with cancer [1,2,18], this raises an interesting question about C/EBPα activity in different lymphocyte subsets in cancer.

PU.1 (Spi1, E26-transformation-specific (ETS) factor) is another transcription factor requiring for the development of the immune system that functions at both early and late stages of lymphoid and myeloid differentiation [19]. The *Pu.1* gene specifically expressed in the early stages of hematopoiesis and its knockout generates mice that lack both myeloid and lymphoid cells [20]. PU.1 mRNA and protein expression is detectable in HSCs, mature monocytes, granulocytes, B, NK cells and pre-T cells [21-23]. Low-level expression of PU.1 in hematopoietic precursors induces B cell differentiation, whereas high levels favor myeloid differentiation [24]. Interestingly, the differentiation potential of T lineage cells becomes restricted soon after entry of multipotent precursors into the thymus and is accompanied by a downregulation of C/EBPα and PU.1 [25].

Furthermore, C/EBPα cooperates with PU.1 to regulate myeloid gene expression [26]. For instance, in pre-B cells, C/EBPα binds to both pre-existing enhancers occupied by PU.1 and *de novo* enhancers, and the combined activation of these enhancers recapitulates the activation of myeloid enhancers and associated genes during normal hematopoiesis [27]. Similar results were obtained in experiments with inducible expression of C/EBPα and PU.1 in pre-T cells: PU.1 converted them into myeloid dendritic cells, while C/EBPα induced the formation of macrophages, which required endogenous PU.1 [25]. Moreover, Notch signaling induced T lineage restriction by downregulating C/EBPα and PU.1 in multilineage precursors [25]. These results together with the findings of altered Notch 1 and Notch 2 expression in NK cells in patients with cancer [2,18] justify further analysis of C/EBPα and PU.1 transcription factors in lymphocytes and hematopoietic precursors isolated from cancer patients.

BACH1 and BACH2 (BTB and CNC homology) are transcription repressors that belong to the basic region-leucine zipper family [28]. With the ability to bind to heme, BACH1 and BACH2 are important in maintaining heme homeostasis in response to oxidative stress [29]. BACH1 and BACH2 promote the differentiation of common lymphoid progenitors to B cells by repressing myeloid-related genes [30]. *BACH1*$^{-/-}$*BACH2*$^{-/-}$ double knockout mice show erythropoiesis disorders with increased myelopoiesis from common myeloid progenitors [31]. BACH2 has critical roles in both acquired immunity and innate immunity, including early B cell development and immunoglobulin class-switch recombination, the development of effector and regulatory T cells and the activation of tissue macrophages [32-35].

BACH1 and BACH2 have important, non-redundant functions in mature myeloid and lymphoid lineages, which are due to partial mutual exclusivity in their patterns of expression: BACH2 mRNA is mostly expressed in mature lymphoid lineages, whereas BACH1 mRNA is generally

expressed in myeloid lineages [36]. Intriguingly, BACH2 represses C/EBPβ and C/EBPβ represses BACH2, and both of them bind to overlapping regulatory regions at their myeloid target genes, suggesting the presence of a mutual feedforward loop leading to myeloid gene regulation [37]. Although, the involvement of BACH2-C/EBPβ pathway in the HSC commitment to myeloid and lymphoid lineages at normal conditions and after infection has thus been shown, little is known about changes of BACH and C/EBP transcription factors in peripheral blood hematopoietic precursors and leukocytes in patients with different types of solid tumors.

The studies described in this report continue to investigate the state of transcription factor expression in immune cells and their precursors in patients with cancer. Our results revealed a significant down-regulation of PU.1 in NK cells and BACH1 in T cells in all cancer patients. However, C/EBPα decrease was detected in monocytes in patients with gastric cancer and neutrophils in patients with lung cancer. Unexpectedly, we revealed a significant increase in the expression of C/EBPα, PU.1 and BACH1 transcription factors in HSC in patients with lung but not gastric cancer. These results demonstrate that abnormal transcriptional regulation of hematopoietic stem cells and differentiated immune cells is cell and cancer dependent and provide new insights into the potential roles of different immune transcriptional factors in tumor escape mechanisms. Although these results are promising, further investigation is justified to identify possible factors responsible for altered expression of transcriptional factors in immune and stem cells in patients with cancer.

Results

Decrease in the PU.1 expression in NK cells in cancer patients

Intracellular expression of C/EBPα, PU.1 and BACH1 transcription factors was determined in the peripheral blood NK cells isolated from patients with lung and gastric cancer and healthy donors utilizing specific antibodies and flow cytometry. MFI data revealed that the expression of PU.1 transcription factor in NK cells in patients with lung cancer (5.55 ± 0.42) and gastric cancer (5.81 ± 0.32) was significantly reduced compared with the expression of PU.1 in NK cells harvested from healthy volunteers (6.99 ± 0.37) ($p<0.003$ and $p<0.02$, respectively) (Figure 1). At the same time, expression of C/EBPα and BACH1 transcription factors in NK cells in cancer patients was not different from their expression in NK cells from healthy donors.

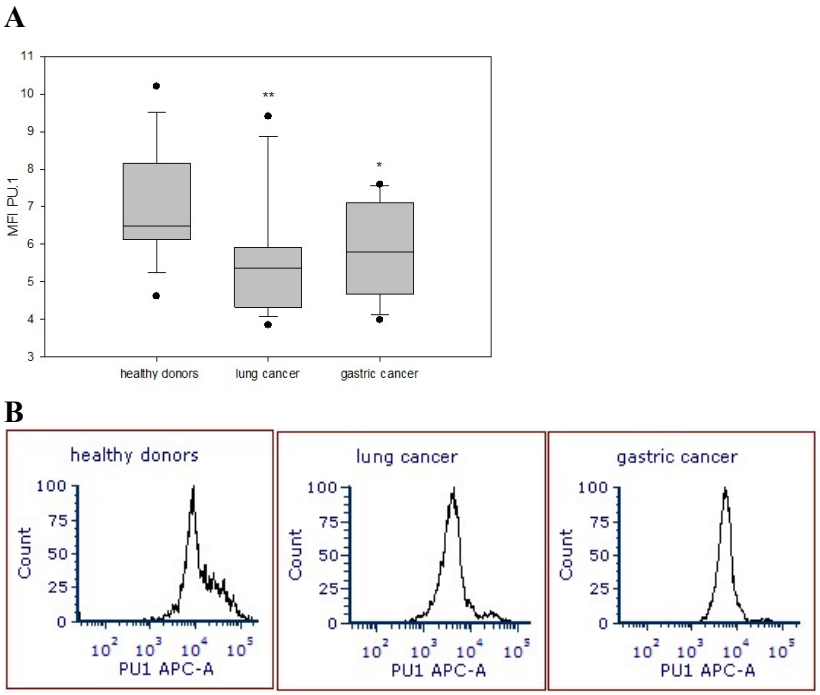

Figure 1: Differences in PU.1 expression in NK cells isolated from healthy donors and patients with cancer. NK cells were isolated from the peripheral blood samples by negative selection using Dynabeads as described in M&M. The relative PU.1 expression was determined by flow cytometry on stained NK cells from healthy donors and patients with lung and gastric cancer. (**A**). results of the mean fluorescent intensity (MFI) are shown as the mean ± SEM (N=14-15). *, $p < 0.05$; **, $p < 0.01$; one-way ANOVA. (**B**). The results of flow cytometry data from a representative experiment are shown.

Reduced BACH1 expression in T cells in cancer patients

Expression of C/EBPα, PU.1 and BACH1 transcription factors also was determined in the peripheral blood T cells isolated from patients with lung cancer and gastric cancer and healthy donors. In contrast to NK cell results, in T cells, only expression of BACH1 was significantly diminished in all patients regardless the type of cancer, tumor cell differentiation stage, clinical stage of the disease or the presence of metastases. MFI data revealed that the expression of BACH1 transcription factor in T cells in patients with lung cancer (3.43 ± 0.13) and gastric cancer (3.99 ± 0.24) was also significantly reduced compared with healthy volunteers (4.98 ± 0.31) ($p<0.001$ and $p<0.02$, respectively) (Figure 2).

Differential expression of transcription factors in monocytes in patients with different cancer localization

The expression of transcription factors in the peripheral blood monocytes was also analyzed. C/EBPα transcription factor expression in these cells was significantly reduced in patients with gastric cancer (8.77 ± 1.27) but not lung cancer compared with the expression seen in healthy volunteers (20.23 ± 3.51) ($p<0.006$) (Figure 3). With respect to transcription factors PU.1 and BACH1, their expression was not significantly reduced in any cancer patient tested cohorts when compared with the results obtained from healthy donors.

Expression of transcription factors in neutrophils

Next, analyzing expression of transcription factors in neutrophils, we revealed a common trend of their down-regulation in cancer in comparison to healthy cells. However, this decrease reached a significant level only for C/EBPα expression in neutrophils obtained from patients with lung cancer (15.48 ± 1.74) compared with healthy volunteers (21.43 ± 2.05) ($p=0.043$) (Figure 4). The expression of BACH1 and PU.1 transcription factors in neutrophils in cancer patients was not significantly altered when compared with healthy donors.

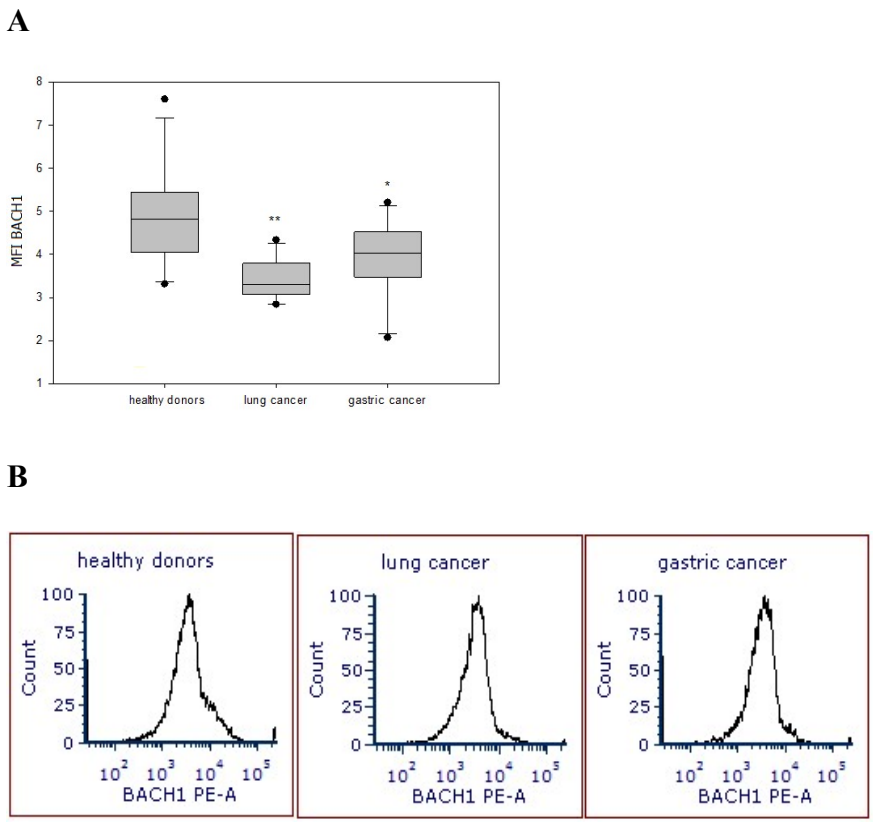

Figure 2: **Differences in BACH1 expression in T cells isolated from healthy donors and patients with cancer**. T cells were isolated from the peripheral blood samples by positive selection using Dynabeads as described in M&M. BACH1 expression was determined by flow cytometry on stained T cells from healthy donors and patients with lung and gastric cancer. (**A**). Results of the mean fluorescent intensity (MFI) are shown as the mean ± SEM (N=14-15). *, $p < 0.05$; **, $p < 0.01$; one-way ANOVA. (**B**). The results of flow cytometry data from a representative experiment are shown.

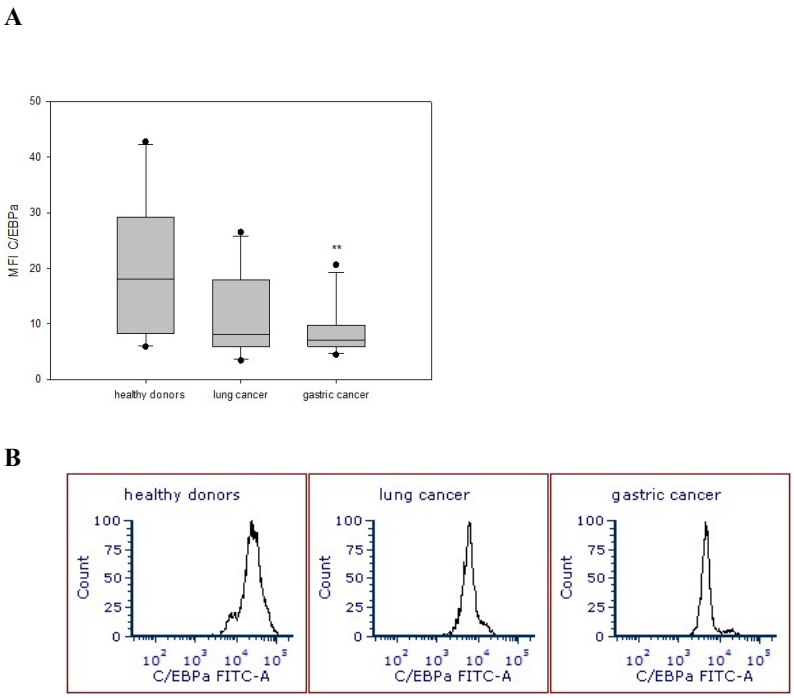

Figure 3: **Differences in C/EBPα expression in monocytes isolated from healthy donors and patients with cancer**. Monocytes were isolated from the peripheral blood samples by positive selection using Dynabeads as described in M&M. C/EBPα expression was determined by flow cytometry on stained monocytes from healthy donors and patients with lung and gastric cancer. (**A**). Results of the mean fluorescent intensity (MFI) are shown as the mean ± SEM (N=14-15). **, $p < 0.01$; one-way ANOVA. (**B**). The results of flow cytometry data from a representative experiment are shown.

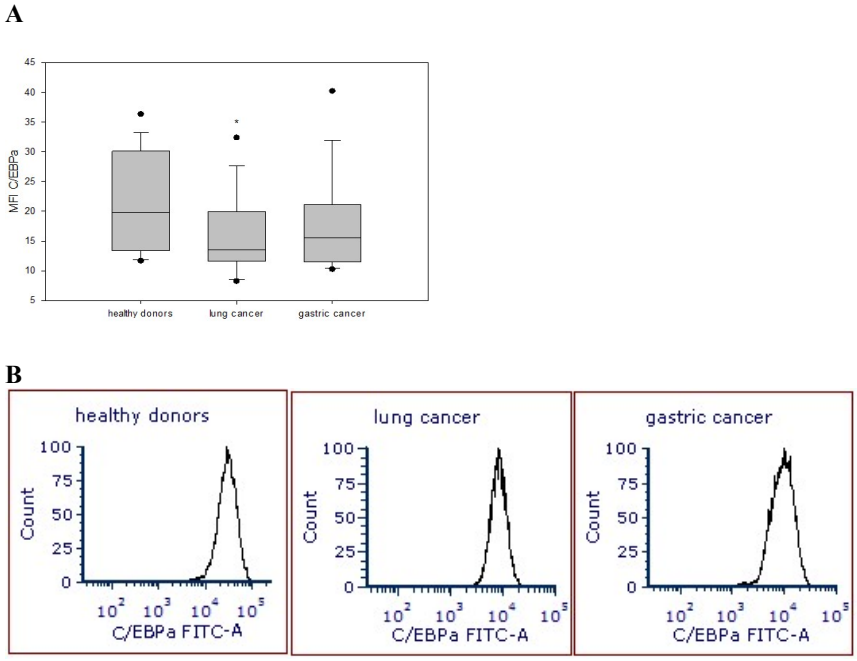

Figure 4: **Differences in C/EBPα expression in neutrophils isolated from healthy donors and patients with cancer**. Neutrophils were isolated from the peripheral blood samples by negative selection using Dynabeads as described in M&M. C/EBPα expression was determined by flow cytometry on stained neutrophils from healthy donors and patients with lung and gastric cancer. (**A**). Results of the mean fluorescent intensity (MFI) are shown as the mean ± SEM (N=14-15). *, $p < 0.05$; one-way ANOVA. (**B**). The results of flow cytometry data from a representative experiment are shown.

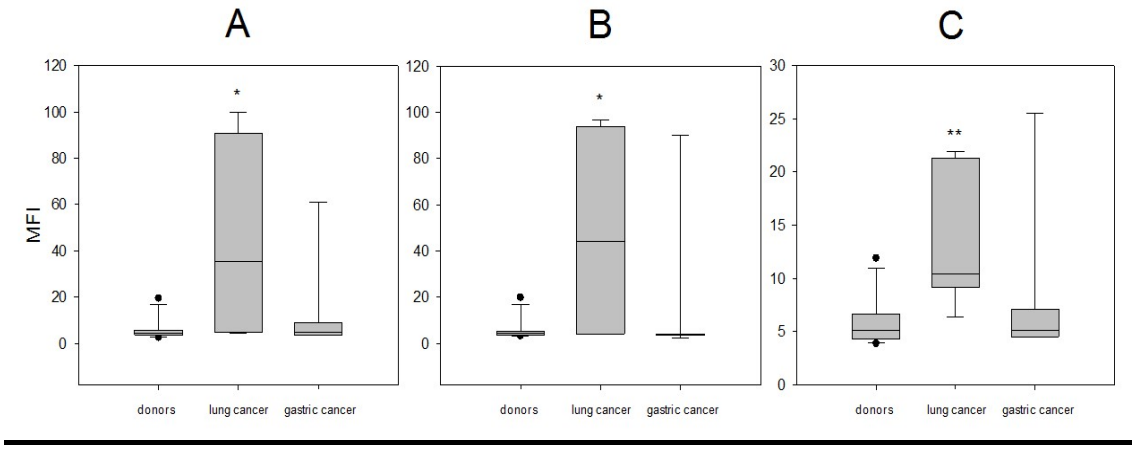

Figure 5: **Differences in C/EBPα, BACH1 and PU.1 expression in stem/progenitor CD34^{+}CD45^{dim} cells isolated from healthy donors and patients with cancer**. CD34^{+}CD45^{dim} cells from the peripheral blood samples were gated based on surface markers. C/EBPα, BACH1 and PU.1 expression was determined by flow cytometry on stained CD34^{+}CD45^{dim} cells from healthy donors and patients with lung and gastric cancer. (**A**). Results of the mean fluorescent intensity (MFI) of C/EBPα are shown as the mean ± SEM (N=6-11), *, p = 0.021, one-way ANOVA. (**B**). Results of the mean fluorescent intensity (MFI) of BACH1 are shown as the mean ± SEM (N=6-11), *, p = 0.044, one-way ANOVA. (**C**). Results of the mean fluorescent intensity (MFI) of PU.1 are shown as the mean ± SEM (N=6-11), **, p = 0.003, one-way ANOVA.

Up-regulation of the expression of transcription factors in CD34^{+}CD45^{dim} cells

CD34 is a transmembrane glycoprotein expressed on early lymphohematopoietic stem and progenitor cells and CD34^{+}CD45^{dim} cells are early stem/progenitor cells [38,39]. Analysis of these cells in the peripheral blood of cancer patients and healthy donors demonstrated a significant increase in the expression of all three transcription factors in patients with lung cancer. For instance, the MFI levels of C/EBPα (44.69 ± 18.35), BACH1 (47.64 ± 19.41) and PU.1 (13.37 ± 2.65) in stem/progenitor cells from patients with lung cancer were significantly higher than in healthy volunteers: C/EBPα (5.63 ± 1.43), BACH1 (5.59 ± 1.44) and PU.1 (5.89 ± 0.68) ($p=0.021$, $p=0.044$ and $p<0.003$, respectively) (Figure 5). No significant differences were detected in transcriptional factor expression in stem/progenitor cells from patients with gastric cancer.

Discussion

Lineage-instructive transcription factors trigger and subdue cell-specific genes by identifying sequence-specific DNA consensus motifs enclosed within enhancers and promoters [40]. Though, how these processes are matched in early and late myeloid and lymphoid differentiation in the tumor environment remain unclear especially as neither transcription factor-induced lineage conversions nor MPP/HPC reprogramming seem to repeat normal differentiation paths during carcinogenesis. Whereas lymphoid and myeloid effector cells possess undisputable tumor-suppressive properties across multiple types of cancer, it is well known that their antitumor properties and function are compromised in cancer patients [41-44]. Established dependence of immune cell development on given transcription factors allows speculating that in the tumor or even pre-tumor environment abnormal functioning of immune effectors is the result of an aberrant differentiation of hematopoietic precursors due to a compromised regulation of transcription factors.

Our previous results revealed multiple defects in c-kit/SCF signaling and c-Myc expression in NK and T cells as well as reduced Notch signaling in NK cells in cancer patients [1,45]. This suggests that a decrease in proto-oncogene expression may be a consequence of a defect in transcription factors associated with their regulation. The c-kit and its ligand SCF play an important role in survival and cytotoxicity of NK cells and differentiation of NK cells from the precursors is reduced in the absence of c-kit signaling [46]. Furthermore, PU.1 plays an intrinsic role in NK cell commitment from lymphoid progenitor, regulates cell development and production of IFN-γ [47], and a significant down-regulation of the c-kit expression in PU.1 deficient NK cells has been reported [48]. In addition, the fraction of cycling NK cells is significantly decreased in *PU.1*$^{-/-}$ chimeras [48]. We have also described a mitotic arrest of NK cells in G0/G1 phase of the cell cycle and decreased amount of NK cells in phases G2/M and S in cells isolated from patients with lung and gastric

cancer [1]. Our new results here revealed a significant reduction in PU.1 expression in NK cells from patients with cancer, which correlates with the previously reported down-regulation of c-kit expression and cell mitotic arrest in the same cells. Therefore, it is possible that previously described molecular abnormalities in NK cells in cancer reflect a single regulatory or developmental pathway that is primarily disrupted in cancer patients.

Our results here demonstrated similar alterations of other transcription factors in isolated lymphocytes. For instance, expression of BACH1, playing an important role in T cell development [37,49], was significantly lower in T cells in cancer patients. Together with the indications of T cell exhaustion in cancer patients [50] and reported down-regulation of c-myc expression in T cells from patients with cancer, which can lead to metabolic defects in T cells [1,51], our new results support the notion about a potential transcriptional dysregulation in lymphoid cells and their precursor cells during carcinogenesis. Further, a growing number of evidences demonstrating that an increased peripheral neutrophil to lymphocyte ratio significantly associated with poorer outcome in various cancers [52-54], our results raised a question about transcription factor expression in myeloid cells in cancer.

The network governing neutrophil-macrophage progenitor cell fate has several key determinants, such as myeloid master regulators C/EBPα and PU.1. Here, we observed decreased expression of granulo-monocytic transcription factor C/EBPα in both monocytes and neutrophils isolated from patients with cancer, while no significant alterations of expression of BACH1 and PU.1 were determined. Interestingly, a decrease in C/EBPα expression in monocytes was observed only in patients with gastric cancer while in neutrophils it was only seen in patients with lung cancer. Although there is no clear explanation for this phenomenon, one can suggest that C/EBPα expression is affected on the level of neutrophil-monocyte progenitor phenotype. However, this ectopic neutrophil-monocyte potential can be transcriptionally or epigenetically reprogrammed in a cancer-specific manner defining the lineage-specific identity of the resulting myeloid malformation. Interestingly, down-regulation of C/EBPα may be induced epigenetically by a lysine-specific demethylase-1 (LSD1) known to be crucial for human leukemogenesis and currently being one of the emerging therapeutic targets [55]. Pharmacological inhibition of factors which control balancing of lineage-specific transcription factors, like LSD1, for instance, should cause lineage-dependent metabolic remodeling in immune cell differentiation. This should open new opportunity in harnessing development of myeloid effector and regulatory cells in the tumor milieu.

Nonetheless, more regulators remain to be identified and characterized under the tumor growth conditions. Another possibility is to characterize transcriptional regulation in the early progenitor cells isolated from cancer patients. Here, we have tested the $CD34^{+}CD45^{dim}$ stem//progenitor cells in the peripheral blood and obtained unexpected results. The up-regulated expression of all tested transcription factors in $CD34^{+}CD45^{dim}$ cells was identified in patients with lung cancer. A particularly high increase was observed in four patients with combined lung and colon or breast cancers and metastases (data not shown). These intriguing results require further verification in bigger cohorts of patients with different types of cancer.

Although C/EBPα plays a well-characterized role in the formation of myeloid progenitors [14] and elicits a lineage-instructive function in MPPs towards the myeloid lineage [12], BACH1/2 regulate both commitment and on-demand hematopoiesis because they control erythroid-myeloid and lymphoid-myeloid differentiation by repressing the myeloid program [56], and PU.1 displays indispensable and distinct roles in hematopoietic development through supporting HSC self-renewal and commitment and maturation of myeloid and lymphoid lineages [57], very little is known about the biological and clinical significance of these transcriptional factor natural up-regulation in hematopoietic progenitor/stem cells especially during carcinogenesis. TNF-α is the principal PU.1 inducing signal in HSCs: it can directly and rapidly upregulate PU.1 protein in HSCs *in vitro* and *in vivo* and is both sufficient and required to relay signals from inflammatory challenges to HSCs [58]. M-CSF (also called CSF1), a myeloid cytokine released during inflammation, can also directly induce activation of the PU.1 promoter and instruct myeloid cell-fate change in HSCs *in vitro* and *in vivo* [59]. Although genetic changes in transcription factor balance are known to sensitize HSCs to cytokine instruction, the initiation of HSC commitment is generally thought to be prompted by stochastic variation in cell-intrinsic regulators such as lineage-specific transcription factors, leaving cytokines to ensure survival and proliferation of the progeny cells. However, the abovementioned facts demonstrate that HSCs are susceptible to direct lineage-specifying effects of cytokines. Therefore, it is possible that tumor/stroma-derived factors may directly alter transcription factors in the early hematopoietic progenitors, as we detected in our studies.

In summary, our results demonstrate altered expression of different transcription factors in lymphoid and myeloid cells in patients with different types of cancer. These changes may be associated with internal dysregulation of hematopoietic stem/progenitor cells induced by early or progressing tumors. The origin and link between these phenomena remain to be determined.

Materials and Methods

Patients' samples

Peripheral blood samples were collected from patients with lung and GI cancers (Table 1). Blood specimens were collected prior to all surgical or chemotherapy procedures. Healthy controls (n = 23), nine men and fourteen women with median age 54 (range 42–68) were recruited from the personnel of the laboratories of the Research Institute of Fundamental and Applied Medicine. All studies were approved by the Institutional Review Board and all patients and healthy donors provided signed written informed consent for sample acquisition for research purposes.

Table 1: Patient demographics.

Characteristics	
Total number	43
Gender, N (%)	
Male	25 (58%)
Female	18 (42%)
Age, years	
Median	62
Range	23-82
Prior anticancer therapies	0
Lung cancer	20 (46%)
NSCLC	17 (85%)
Adenocarcinoma	15 (75%)
Squamous cell carcinoma	2 (10%)
GI cancer	23 (54%)
Esophageal ADC	6(26%)
Colorectal cancer	6 (26%)
Sigmoid cancer	2 (8.6%)
Gastric ADC	9 (39.1%)
Metastasis	
Primary tumor site	Localization of metastasis
Lung ADC (4)	Bones, colon, breast, mediastinum
Colorectal cancer (3)	Lymph nodes

Isolation of NK cells, $CD3^+$ T cells and monocytes

PBMC were isolated from the peripheral blood by Ficoll-Paque PLUS (Life Technologies, USA) density gradient centrifugation. NK cells were negatively selected using DynaMag Magnet with Dynabeads Untouched Human NK Cells Isolation kit (Life Technologies). T cells were isolated from PBMC by positive selection using DynaMag Magnet with Dynabeads Untouched Human CD3 microbeads (Life Technologies). Monocytes were isolated from PBMC by positive selection using DynaMag Magnet with Dynabeads™ CD14 microbeads (Invitrogen). The purity of the cell subsets was confirmed by flow cytometry (FACSCanto II, BD Biosciences) using specific CD56 and CD16 monoclonal antibodies conjugated with FITC and PerCP, specific CD3 monoclonal antibodies labeled with FITC and specific CD14 monoclonal antibodies labeled with PE (BD Biosciences). Data analysis was performed with BD FACSDiva Software.

Isolation of neutrophils

Neutrophils were isolated using the method that allows fast isolation of untouched neutrophils without density gradient centrifugation. Erythrocytes in the peripheral blood samples were aggregated and sedimented and neutrophils were removed by immunomagnetic purification with MACSxpress Beads (MACSxpress® Whole Blood Human Neutrophil Isolation Kit, Miltenyi Biotec Inc.). Phenotype of neutrophils was confirmed with CD15 expression by flow cytometry. All experimental procedures were optimized using blood samples from healthy donors.

Gating strategy for cell analysis

Gating strategy for NK cell analysis: the first step is cutting off dead cells on the FSC, then, in the area of lymphocytes, NK cells were identified based on FSC/SSC. The purity of isolated NK cells after isolation and enrichment procedures was in the range of 89-95%.

Gating strategy of CD3 positive cell analysis: the first step is cutting off dead cells on the FSC, second step - in the area of lymphocytes; T cells were identified based on FSC/SSC. The purity of isolated CD3 cells after isolation and enrichment procedures was in the range of 93-96%.

Gating strategy of monocyte cell analysis: the first step is cutting off dead cells on the FSC, then monocytes cells were identified based on FSC/SSC and CD14 expression in the area of monocytes. The purity of the isolated monocytes after isolation and enrichment procedures was within the range of 87-94%.

Gating strategy of neutrophils cell analysis: the first step is cutting off dead cells on the FSC, and then neutrophils were identified based on FSC/SSC and CD15 expression in the area of granulocytes. The purity of the isolated neutrophils after isolation and enrichment procedures was within the range of 90-93%.

Gating strategy of CD34CD45dim cell analysis: the first step is cutting off dead cells on the FSC, cells

are gated in the CD45dim-lymphocyte area, then 7-aminoactinomycin D (7-AAD)-positive dead cells were separated from viable CD34 positive 7-AAD-negative cells.

Detection of C/EBPα, PU.1 and BACH1 protein expression

For the intracellular detection of C/EBPα, PU.1 and BACH1 transcription factor expression by flow cytometry, isolated NK cells, T cells, neutrophils and monocytes were fixed, permeabilized and stained with differentially conjugated mouse monoclonal anti-human C/EBPα, PU.1 and BACH1 antibodies: C/EBPα (D-5) Alexa Fluor® 488 sc-365318 AF488, 200 µg/ml (RUO), BACH1 (F-9) PE (RUO), PU.1 (C-3) Alexa Fluor® 488, sc-390405 AF488 200 µg/ml (RUO) (Santa Cruz Biotechnology, Inc.).

Expression of transcription factors in the stem/progenitor cells

Expression of C/EBPα, PU.1 and BACH1 was determined in the peripheral blood cell samples gated on $CD34^{+}$ $CD45^{dim}$ cells by flow cytometry using conjugated mouse monoclonal anti-human C/EBPα, PU.1 and BACH1 antibodies according to the manufacturer's protocols. Cells were analyzed by a 10-color FACSCanto (Becton Dickinson). CD45 was used as a marker of blood hematopoietic cells to exclude mature erythrocytes and platelets. $CD34^{+}CD45^{dim}$ cells were considered stem/progenitor cells [60,61]. CD34 PE-Cy7 and CD45 PerCP-Cy5 antibodies were used for cell phenotyping. Data analysis was performed with BD FACSDiva Software.

Statistical analysis

For a single comparison of two groups, the Student's *t*-test was used after the evaluation of normality. If data distribution was not normal, a Mann-Whitney rank sum test was performed. For the comparison of multiple groups, analysis of variance (ANOVA) was applied. SigmaStat Software was used for data analysis (SyStat Software Inc.). For all statistical analyses, $p<0.05$ was considered significant. All experiments were repeated at least two times. Data are presented as the mean ± SEM.

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Author Contributions

Conceptualization, writing, original draft preparation, Gulnur K. Zakiryanova; methodology, project administration, supervision, Narymzhan N. Nakisbekov; formal analysis, investigation, data curation, Elena Kustova, Nataliya T. Urazalieva, Galina V. Shurin; resources, Emile T. Baimuchametov, Valeriy A. Makarov; writing, review and editing, visualization, funding acquisition, Michael R. Shurin.

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Conflicts of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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