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Expressions of CD80 and CD86 in Cancer Patients and Its Prognostic Significance

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Abstract **Background:** Strategies for modulating the tumor microenvironment (TME) have opened up new treatment paths in a variety of malignancies, with dramatic but variable intertemporal success. Consequently, studying TME's molecular players may aid in understanding how tumor cells and TME interact. Tumor cells and infiltrative tumor lymphocytes express immune checkpoint proteins including Cluster of Differentiation 80 (CD80) and CD86 on their surface. CD80 and CD86 are members of the immunoglobulin superfamily (IgSF). The costimulatory protein CD28, which is present on the outermost portion of all T cells, and the inhibitory receptor CTLA-4 (cytotoxic T-lymphocyte antigen-4, also known as CD152) are ligands for CD86 and CD80. CD28 and CTLA-4 have essential yet opposing functions in T cell activation. T cell responses are stimulated when they bind to CD28, but they are suppressed when they connect to CTLA-4. **Aim of the study:** The goal of the study was to determine how much the CD80 and CD86 genes were expressed genetically. In five blood malignancies and ten solid tumors. **Methods:** Clinical specimen were collected from patient with different cancers attending Kirkuk oncology centre. Patients were categorised into two main groups: solid tumour group and Blood derived cancer group alongside with their control counterparts. The study investigated the genetic expression of the CD80 and CD86 genes using q RT-PCR technologies. **Results:** Using q RT-PCR we measured the expression of a gene CD80 /CD86. The results showed different levels of elevation in patient samples of solid tumors and hematological tumors and compared with the control group for this study First, evaluation of this marker CD80/CD86 in solid tumors showed a significant increase in patients with brain cancer compared to their counterparts in the control group. Secondly, the second solid tumor appears increased gene expression as ovarian cancer. The least expressed solid tumors are breast cancer. As for cancers that occur in the blood, lymphoma has an upregulation expression. And the least expression is CD80/CD86 were in ALL. **Conclusion:** There is evidence that several cancer types and immune cells have expressed CD80 and CD86. This investigation demonstrated that the cell surface markers CD80/CD86 have a role in progression of carcinomas of the brain.

Key Words CD80 , CD86 , solid tumors, brain cancer, immunological checkpoint proteins

1. Introduction

Cancer is considered one of the most health threatening issues in the world, therefore, understanding its pathology and the mechanistic pathways involved in its spread is an essential way to tackle this problem Cancer cases is growing more and more in Iraqi population recently [1]. According to the annual report of Iraqi cancer registry during 2021, the highest cancer incidence was reported in the city of Baghdad Governorate in both genders in female No. 5255 – 14.67% or male No. 3477 – 9.71% The least of them are in Al-Muthana Governorate female No. 335 – 0.94% or Male No. 276 – 0.77% Kirkuk had the lowest infection rate female No. 614 –1.71% or male No. 445 –1.24 %.

Tumor and immune cell surfaces contain immunological checkpoint proteins including Cluster of Differentiation 80 (CD80 also referred to as B7-1) and CD86 (also referred to as B7-2).T cell activation is regulated by B7 superfamily members B7-1 (CD80) and B7-2 (CD86). belong to the immunoglobulin (Ig) superfamily and are both type- 1 proteins play a role in the initiation and maintenance of immune responses to both internal and external invaders, including cancer cells [2].

T-lymphocytes carry the markers Cluster of Differentiation 28 (CD28) and Cytotoxic T-lymphocyte-associated antigen–4.The major histocompatibility complex and antigen-presenting cells have ligands that both the CD28 and CTLA-

4 proteins bind to. T-cell function is activated or inhibited by immune checkpoint proteins interacting with their co-receptor on the surface of T-lymphocytes. For example, CTLA-4 binds to CD80 and CD86 more strongly than other immune checkpoint proteins, causing T cells to become fatigued [3].

CD80/86 molecules regulate immunological microenvironment in cancer tissues. Hematologic cancers and a variety of solid cancers, including glioma, gastric cancer, and pancreatic cancer, have been shown to express CD80 and/or CD86 [4].CD80 (B7-1) is a 33 kDa which is a 288 amino acid immunoglobulin protein encoded by CD80 gene. CD86 (B7-2) is a 70 kDa as a 329 amino-acid immunoglobulin protein encoded by CD86 gene [5].

B7-1 mainly appears as a dimer on activated B cells, activated T cells, and macrophages. On the other hand, CD86 is a monomer that is considered to be more commonly expressed at greater levels than CD80, which has constitutively active expression in dendritic cells (DCs), Langerhans cells, peripheral blood, memory B cells, and germinal center B cells in the body [6].

B7-1 and B7-2 are type -1 transmembrane glycoproteins that have two external Ig-like domains [7].For the onset of adaptive immunological responses, both CD80 and CD86 are necessary. T cells need to receive two signals in order to activation. Initial signal transmission occurs via the T-cell receptor (TCR), which identifies antigen bound to the major histocompatibility complex (MHC). It is believed that energy or tolerance are caused only by this signal. The 'stimulatory' or 'accessory' signal is the second one. CD28 is a major mediator of this second signal on T cells [8].

2. Materials and Methods

A. Patients and Samples

Blood samples were collected from patients attending the oncology centre of Kirkuk, Iraq during the period from December, 2022 to June, 2023. All information and consent forms were issued under the supervision of ministry of health regulations. The study included 152 subjects which were divided into two main groups: control group (50 healthy people) and patient group (62 solid tumours, 40 blood cancers).A total of four ml blood samples were obtained from each subject under aseptic conditions, two ml of each blood samples was stored in EDTA tubes at -20 freezer for the gene expression experiments.

B. RNA Extraction

RNA was extracted from each blood samples following manufactures instructions (GENEzol TriRNA extraction Kit, Genoid, cat# GZX050, Korea). Three volumes of GENEzol reagent were first added to 200μl of blood sample in the ratio of 1:3 in order to eliminate erythrocytes and obtain leukocytes only then samples were vortexed and incubated 5 min RT, centrifuged at 12000xg for 1 min then samples were taken to new micro centrifuge tubes and one volume of absolute ethanol was added and mixed by vortex and then RB columns were prepared and fixed to collection tubes. This was followed by transferring samples to the RB columns, centrifuged for 1 min at 14000 xg the flow-through was eliminated and the filtered column placed back on the collection tube, Then three steps of RNA washing by adding 400μl,600μl, 600μl wash buffer and centrifuged for 30 sec. at 14000 xg between each wash, then the flow through were discarded and the RB column were placed in new collection tubes where 25 μl of RNAase free water was added to each column and left for 3 min at RT then centrifuged at 14000 xg and eluted samples were kept at -20 until use .

C. Gene Expression

RNA samples were then used for the gene expression study. GoTaq 1-Step RT-qPCR Kit, Promega, cat# A6020 Kit was used for q RT-PCR and specific primers were designed for CD80 gene from NCBI PRIMER BLAST tools were forward primer 5'-GAAGAGCTGGCACAAACTCG-3'and reverse primer 3'-CGCAGAGCCAGGATCACAAT-5'. CD86 gene forward primer 5'-CAGACCTGCCATGCCATTT-3' and reverse primer 3'-CCTGTCAACCTGGGACTCTG-5'. B globin gene was used as a reference gene (Forward 5'-ACACAACTGTGTTCACTAGC-3', Reverse 5'-CAACTTCATCCACGTTCACC-3'). The fold change of the relative mRNA was calculated using the comparative threshold method (ΔCt control= Ct target gene (CD80/ CD86 gene) - ct reference gene(β-globin), Δct patients= Ct target gene (CD80/CD86 gene) - ct reference gene (β-globin), $\Delta\Delta$ct = Δct patients- ΔCt control and the fold change = $2^{-\Delta\Delta ct}$ [9] . PCR conditions were initial denaturation 95 $^{\circ}C$ for 2 min, (denaturation 95 $^{\circ}C$ for 30 sec, annealing 55 $^{\circ}C$ for 30 sec and extension 72 $^{\circ}C$ for 40 sec) 40 cycles using q Tower3 G, Germany.

D. Statistical Analysis

All data presented in this article was performed using Graph pad prism software. Data represented M±SEM and significance of differences were tested between all groups using one – way analysis of variance (ANOVA) with Bonferroni's multiple comparison test.

3. Results

A. Variations in the Expression of CD80 at the mRNA Level in Hematological and Solid Tumor Types

We have determined the patterns of expression for CD80 in a variety of cancer types, such as hematological and solid Malignancy. We evaluated the cancer cell types in individuals who expressed high and low levels of both CD80 and CD86. Tumor-associated macrophage surface expression of CD80 and CD86 suggests a role in immunosuppressive. As shown in Figure 1 below, we found expression of CD80, specifically in solid tumors such as brain cancer (7450±2747), which had the highest expression, while the other types were Expression of CD80 is somewhat similar. In breast cancer (124.333±2.963) Colon cancer was (359.670±103.475) ,Ovarian (284.670±271.147)

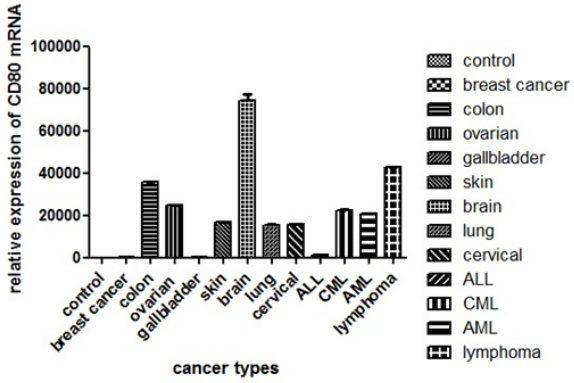

Figure 1: Expression of CD80 in type of cancer

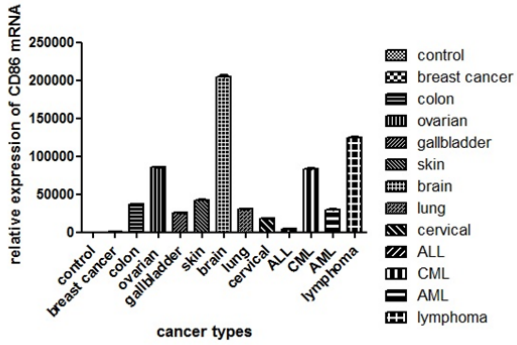

Figure 2: CD86 upregulation in solid and blood cancer

, Bladder Cancer (149.667±4.485) ,Skin (166.330±197.404) ,Lung(155.330±318.313),In cervical cancer(157.330±84.887).As for hematological tumors, lymphoma had the highest expression among the types of blood cancers (426.000±303.009) Than acute lymphatic leukemia (ALL) (118.333±33.716),chronic myeloid leukemia was (224.330± 331.432), Acute myelogenous leukemia (AML)(207.670±139.957) All was significantly increased ($p < 0.001$) when compared to their controls.

B. CD86 Gene Expression Differs Between Cancers

Results of CD86 in q RT-PCR experiments showed different levels of elevation in this study. First, evaluation of this marker in solid tumour showed a dramatic upregulation ($p<0.05$) of CD86 fold change in patients with brain cancer (203982 ± 3505) compared to their control counterparts. Second solid tumour to show increased CD86 gene expression was ovarian cancer (86278.12 ± 161.95), followed by skin cancer (42498.81 ± 478), colon cancer (36650 ±607.70), lung cancer (30476.62 ± 376), gallbladder cancer (25713.45 ± 464.17), cervical cancer (18.045.94 ± 84.49), and the least fold change recorded was in the breast cancer (304 ± 5.15).

Results of CD86 mRNA levels in blood cancer show significant upregulation in lymphoma patients (125162±238.4) at $p<0.05$,second blood cancer to show increased CD86 expression was CML patients(83755.94±503.66), followed by AML patient where showed (29916.16±400.45) fold change. While ALL patients showed the least expression (4835.7±233.72) among other blood cancers but still was significantly increased when compared to their controls as shown in Figure 2 .

4. Discussion

Previous research has shown that it has been demonstrated that multiple types of cancer have expressed CD80 (Golubovskaya, 2022). Prior research has demonstrated that the expression of CD80 is reduced in several types of cancerous cells, and that CD80 depletion alone enhances the ability of these cells to avoid immune system attacks and gives tumor-infiltrating T cells vigor and apoptosis [10].

According to previous research by [11], suggesting the CD80 expression levels may control the pro- or anti-oncogenic effects of CD80 on tumor cells. Because CTLA-4 binds to CD80 more preferentially than it does to CD28, low levels of CD80 expression provide a pathway for tumor escape from immune surveillance. Conversely, CD80 overexpression encourages T cell activation and tumor rejection, while CD80 deficiency also makes tumor cells more immunogenic.

CD86 is a cell surface marker that is expressed by antigen presenting cells and interact with its receptor that is called cytotoxic T-lymphocyte associated protein-4 (CTLA-4) and this reaction regulate the activity of T-cell in many cancer types [4]. Results of the current study showed significant increase in the CD86 mRNA in brain cancer as mentioned in the previous paragraph. In this regard, recent evidence from other researcher showed that CD86 alongside with CD80 both are associated with shorter progression free survival [3]. Others suggested that CD86 –dependent signalling inhibition as a promising therapeutic strategy for immunotherapy in many tumour [6]. Another study conducted by [12] reviled that patient with increased CD86 gene expression showed worse overall survival than others with less CD86 expression. This indicate that high expression of this marker is linked to the tumor progression and aggressiveness, this idea is consistent with a study conducted by [13] where they found low expression of CD86 in early stages of cancer and increased afterward in late stages. The role of CD86 overexpression by macrophage showed to have an inhibitory effect on T-lymphocyte activation in the tumor microenvironment which lead to poor survival in glioma patients [14]. This might support the current study's result about brain cancer as glioma is one type of brain cancer. However, further investigation is needed at the cancer subtype level, but due to the time limitation was not able to continue this line in this study.

These results are in line with another recent study where they show CD86 gene and protein upregulation in AML cell lines using q RT-PCR and western blotting respectively and they revealed that CD86 overexpression is linked with poor prognosis and negatively linked with immunotherapy due its relation with higher cancer grade and larger tumor size [15].

5. Conclusion

At the mRNA level, we measured the gene expression of CD80 and CD86 Using qRT-PCR technology. The results of this study showed the highest expression of CD80, specifically in solid tumors in brain cancer patients, subsequently colon cancer, then ovarian cancer patients. The lowest expression of CD80 is breast cancer and bile duct cancer. In hematological tumors, lymphoma was significantly increased, and ALL had the lowest genetic expression of the CD80 gene. Regarding CD86 gene expression, his results were It is similar to CD80. Brain cancer also showed high expression of CD86, then ovarian and colon cancer. Also in hematological tumors, the highest expression of CD86 was in lymphoma and the lowest expression in acute lymphoblastic leukemia (ALL). In cancer patients receiving immunotherapy, CD80/CD86 may be a useful biomarker for prognosis. More research is necessary to confirm these preliminary results.

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Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

Concept and design: Shilan Jabbar. Data acquisition and analysis: Athraa Ismael & Shilan Jabbar. Manuscript preparation and revision to finalizing manuscript: Athraa Ismael and Shilan Jabbar.

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