Molecular and Immunological Analysis of the Effect of Tumor Associated Macrophages on Prognosis of Hodgkin’s Lymphoma in Saudi Patients

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Introduction

Hodgkin’s lymphoma (HL) is a malignant lymphoma that most commonly affects younger patients. HL is characterized by a minority of neoplastic cells: Hodgkin’s Reed–Sternberg Cells (HRS). HRS are surrounded by the tumor microenvironment (TME), with frontline tumor associated macrophages (TAMs). The high number of TAMs constitutes a major part of the cellular milieu and was identified as the major determinant of the clinical outcome of HL. Over expression of CD68 and CD163 antigens on TAMs is considered significant predictive biomarker for risk stratification of HL. This is thought to be caused by genetic polymorphism (SNPs) at the promoter or the coding region of their genes. Moreover, common DNA polymorphisms of related DNA repair genes in Saudi patients and their link to disease progression and/or survival can be used as prognostic biomarkers and may help researchers identify a curative therapy for HL. The classical HL (CHL) comprises the majority of all HL cases. A review of records at John Hopkins Aramco Healthcare Center-Dhahran-KSA showed that CHL represents more than 85% of HL cases. Although HL is a relatively curable disease, a considerable percentage of cases (20-30 %) fail to respond to therapy and an equal percentage are over treated with current protocols. Early identification of those cases has become an important objective for clinical research. Even though the epidemiological behavior of HL is continuously updated in various parts of the world, studies from the Kingdom of Saudi Arabia are limited. Recent studies confirmed a molecular/gene signature associated with macrophage infiltration in CHL.

Objectives: The aim of this study was to investigate the significance of expression of CD68 and CD163 antigens on TAMs and the presence of selected SNPs on CD68 and CD163 genes and a transcription regulator gene SNP was performed for 100 CHL patients and normal controls using Real-Time PCR.

Keywords: Hodgkin’s lymphoma, Tumor associated macrophages, Immunohistochemistry, CD30, CD68, CD163, DNA repair genes, Predictive biomarkers.

Methods

This is a retrospective case-control study. The protein expression of CD68 and CD163 on TAMs was studied using Immunohistochemistry Staining (IHC). A prognostic index was calculated for both proteins to assess the risk stratification of HL. We intended to introduce a new methodology for calculating a “Prognosis Index” of CD68 and of CD163 as well. That was calculated by dividing the average number of CD68 positive TAMs surrounding a cluster of HRS by the average number of CD63 HRS positive cells. The equation is as follow:

Data revealed that both CD68 and CD163 created indices are highly correlated to each other. The expression levels of those two proteins were significantly correlated with the disease relapse (OS) and overall survival (OS) with a p-value of $<0.001$ for both. In addition, the CD163 index threshold of $(15.0)$ was significantly correlated with the relapse rate $(p=0.022)$. None of the CD68 gene SNPs were correlated with DR or with OS of HL patients except for n17608120, which is located in the promoter region. This SNP is showing a significant correlation with the relapse rate of HL $(p=0.032)$ but not with OS. The analysis of 5 DNA repair gene SNPs showed that the XPG gene has a statistical significance correlation with HL patients OS $(p=0.032)$. This finding suggests that the XPG gene can be used as a molecular predictive biomarker for HL clinical prognosis.

Conclusions

Through this study we gained valuable insights on the molecular genetics and immunological contribution of CD68 and CD163 on HL in Saudi Arabian patients. We have confirmed that CD68 and CD163 over expression on TAMs is significantly associated with early relapse and reduced survival post therapy, with more specificity of CD63 antigen. Notwithstanding, we have also defined, for the first time, a specific genetic pattern that is clearly associated with the clinical outcome of HL. A specific SNP in XPG DNA repair gene and a SNP located in the CD163 promoter sequence of the gene were both significantly correlated with HL relapse risk and poor overall survival rate. Accordingly, they are argued to be useful predictive immunological and genetic biomarkers for CHL prognosis in Saudi patients.

Moreover, our findings pave the way to improve the clinical management of HL patients in the future using molecular analyses for gene expression and immunohistochemistry. Our findings may help to achieve better therapeutic protocols.

Pharmacogenetics, specifically monoclonal antibodies, could be used to block TAMs receptors for CD68 and CD163 in conjunction with the current chemotherapy or immunotherapy used to treat HL.

Bibliography


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