Genetic Polymorphisms in Cytotoxic T-Lymphocyte Antigen 4 and Cancer: The Dialectical Nature of Subtle Human Immune Dysregulation

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Abstract

Genetic polymorphisms in the human genome are an important component of genotypic variability including one’s immune status. Single nucleotide polymorphisms (SNPs) in the cytotoxic T-lymphocyte antigen 4 (CTLA-4) gene have been linked to susceptibility to autoimmune disease. Interestingly, we have recently shown that an SNP in the CTLA-4 coding region (49A > G) is also associated with susceptibility to human cancer, but the risk allele for susceptibility to cancer (allele A) is the opposite of that found for susceptibility to autoimmune disease (allele G), which has been confirmed by a meta-analysis of reported studies. These findings indicate an important role of the dialectical nature of T-cell immune dysregulation in human disorders, such as autoimmune disease and cancer. The requisites of CTLA-4 polymorphisms for susceptibility to cancer and response to targeted therapy are discussed in this review. [Cancer Res 2009;69(15):6011–4]

Introduction

It has been recognized for many years that humans can initiate a response to developing tumors. Previous studies have shown that dense intratumoral lymphocyte infiltration of early lesions is associated with reduced frequencies of metastasis and improved patient survival (1, 2). It has also been documented that individuals who were immuno-suppressed have an increased risk for tumor development (3). Because cancer cells can only slightly provoke immune recognition, proper activation and function of cytotoxic T lymphocytes (CTLs) are critical for immunosurveillance. The activation of CTLs requires the presence of tumor-derived antigens on the surface of antigen-presenting cells (APCs) having the capacity to provide costimulation. Picking up from tumor cells, APCs process the tumor antigen and present it as a pMHC complex on their surface in the context of B7 costimulation. In the normal process of eliminating foreign biological materials, such as infectious agents, upon T-cell activation, a negative regulatory mechanism immediately functions to terminate unnecessary T-cell expansion to maintain homeostasis (4). Unfortunately, such negative immune regulatory circuits may also suppress antitumor immunity.

Cytotoxic T-lymphocyte antigen 4 (CTLA-4, also known as CD152) plays a pivotal role in the negative regulation of T-cell proliferation and activation. This activation-induced homodimeric glycoprotein receptor on CTLs interacts with B7.1 (CD80)/B7.2 (CD86) ligands on the surface of APCs, resulting in cell cycle arrest and inhibition of cytokine production, which directly eliminates the T-cell proliferation phase (5). Furthermore, CTLA-4 may indirectly control effector T cells by its constitutive expression on T-regulatory cells. As an inhibitory mechanism leading to down-regulation of T-cell response and peripheral tolerance, besides minimizing harm to normal tissues, CTLA-4 is also known to be able to diminish the generation of effective antitumor responses and thus bring tumor tolerance (6). It has been hypothesized that during the early stage of tumorigenesis, CTLA-4 may elevate the T-cell activation threshold, thereby attenuating the antitumor response and increasing cancer susceptibility (6).

Although antitumor responses are found to differ greatly among cancer-bearing hosts, the underlying mechanisms are not well understood. Genetic variations such as single nucleotide polymorphisms (SNPs) can be of functional significance and associated with disease phenotypes. Thus, it is believed that antitumor responses such as proliferation and activation of tumor specific CTLs may also be influenced by genetic polymorphisms. Because genetic polymorphisms in CTLA-4 have been linked to a variety of autoimmune diseases (7), the impact of these polymorphisms on cancer susceptibility has also attracted research interest. In this review, we focus on functional genetic polymorphisms in the CTLA-4 gene and their association with risk of cancer development.

CTLA-4 Polymorphisms and Their Function

Many SNPs have been identified in the CTLA-4 gene that contains three exons and two introns. To date, at least five SNPs that were well studied in immune diseases have been investigated in cancer association studies. Among them, the −318C > T SNP (rs5742909) located in the promoter region, the 49A > G SNP (rs231775) located in exon 1, and the 6230A > G SNP (CT160, rs3087243) located in 3′-untranslated region (3′UTR) have attracted more attention. Several studies have shown that the CTLA-4 −318C > T SNP may have functional significance. The −318T allele has been reported to have higher promoter activity than the −318C allele (8), probably due to creating a lymphoid enhancer factor-1 (LEF1) binding site in the CTLA-4 promoter (9). It has been shown that T cells with the CTLA-4 −318T allele have increased CTLA-4 expression compared with those with the −318C allele, which is believed to play an important role in suppressing antitumor immune activity (10, 11).

The CTLA-4 49A > G SNP causes 17Thr > 17Ala substitution in the leading peptide of CTLA-4 receptor (12). It has been reported that the 49G allele has lower mRNA efficiency and decreased CTLA-4

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production than the 49A allele (9, 13) and, therefore, individuals with the 49GG genotype have higher T-cell proliferation than those with the 49AA genotype under the condition of suboptimal stimulation (14). However, such an effect was not observed in another study (15). We have recently found that the 17Thr > 17Ala change in CTLA-4 caused by the 49A > G SNP greatly enhanced the receptor to interact with its ligand B7.1, and recombinant CTLA-4-17Ala had a significantly stronger ability to inhibit T-cell proliferation and activation compared with its counterpart CTLA-4-17Thr (16). Further experiments confirmed that PBMCs having the CTLA-4 49AA genotype showed a significant decrease in T-cell proliferation and activation compared with PBMCs having the CTLA-4 49GG genotype upon stimulation with PHA (16). These findings indicate that the 17Thr > 17Ala change in CTLA-4 results in stronger CTLA-4-triggered inhibition of T-cell proliferation and activation.

The CTLA-4 6230A > G SNP has been shown to play an important role in the processing and production of soluble CTLA-4 (sCTLA-4), an isoform lacking the transmembrane domain, which is generated by alternative splicing of the primary transcript. The sCTLA-4 may have dual effects on regulation of the immune response. It can bind B7 on APCs to shield the B7/CTLA-4 interaction and impede the negative signal to T cells. Alternatively, it can also block the B7/CD28 signaling to prevent costimulation of T-cell activation (17). It has been shown that the CTLA-4 6230A > G SNP was correlated with lower mRNA levels of sCTLA4 and associated with susceptibility to autoimmune diseases (18).

Taken together, the results presented above indicate that some SNPs in CTLA-4 are biologically functional and might be genetic susceptibility factors for common human cancers.

### CTLA-4 Polymorphisms and Susceptibility to Human Cancer

Although there is a large body of evidence showing that genetic polymorphisms in CTLA-4 are associated with autoimmune diseases (19–21), only a few studies investigating the role of these polymorphisms in human cancer have been published. Because most cancer studies have focused on the CTLA-4 49A > G SNP and the function of this SNP is well verified, we identified all nine studies published before December 2008, and did a meta-analysis of these studies, which includes a total of 14,374 cancer patients and controls (16, 22–29), to examine the association between 49A > G genotype and risk of cancer. The cancer types included in these studies are breast cancer (16, 23, 24), lung cancer (16), esophageal cancer (16), gastric cancer (16, 25), colorectal cancer (25, 26), renal cell cancer (27), oral cancer (28), and cervical cancer (29).

As shown in Fig. 1, the CTLA-4 49A > G SNP is significantly associated with cancer risk compared to the 49AA genotype. The odds ratio (OR) was higher for the 49AG genotype compared to the 49GG genotype, and the difference was statistically significant.

#### Figure 1

Odds ratios (95% CIs) from individual studies testing the association between risk of cancer and CTLA-4 49A > G genotype. The aggregate odds ratio (95% CI) for the AA and AG genotype is also given along with the probability of heterogeneity of the Cochran’s Q test. The weighting factors (Weight %) used to calculate the aggregate odds ratio, calculated from the inverse of the variance, are given for each study.
risk of multiple types of cancer. The reported study with the largest sample size was done by two Chinese populations. Although studies with larger sample size tend to yield smaller variance and more conservative results, the meta-analysis still showed a significant association between the \textit{CTLA-4} 49A > G SNP and susceptibility to multiple types of cancer even without our data set (16), with a pooled odds ratio of 1.36 (95% confidence interval (CI) = 1.08-1.71), using a random-effect model based on the DerSimonian and Laird method and analyzed by the Stata 9.2 software. Although it is difficult to select a genetic model to best fit the effect, the results of our meta-analysis are mostly consistent with an autosomal dominant model: both 49AA and 49AG genotypes are associated with increased risk. Moreover, the results from analysis stratified by ethnic subgroups are consistent. The variant A allele is less frequent in Asians (32%) than in Caucasians (62%), but the genetic effect conferred by the A allele seems consistent in different ethnic populations.

The variant \textit{CTLA-4} 49A allele has been shown to be a protective allele in autoimmune diseases; however, in cancer it is a risk allele associated with elevated susceptibility to multiple types of cancer (Fig. 1). Taking into consideration the role that \textit{CTLA-4} plays in suppressing T-cell activation and the effect of CTLs on immunosurveillance of cancer, this opposite direction in association between \textit{CTLA-4} 49A > G SNP and autoimmune diseases or cancer is biologically plausible. It would be interesting to examine the role of functional \textit{CTLA-4} SNPs in susceptibility to cancer developed from the immune system, such as T-cell leukemia and T-cell lymphoma, in which the variant \textit{CTLA-4} 49A allele might act as a susceptibility factor (30).

\section*{\textit{CTLA-4} Polymorphisms and Cancer Immunotherapy}

The discovery and functional characterization of \textit{CTLA-4} as a key negative regulator in immune response has prompted efforts to develop cancer immunotherapy targeting this signaling molecule (31). Inhibition of \textit{CTLA-4} activity with anti-\textit{CTLA-4} antibody allows T-cell expansion to continue after vaccination with tumor antigen. Although anti-\textit{CTLA-4} blockade alone is less effective against poorly immunogenic tumors, combination therapy with vaccines generates impressive therapeutic effects. Preclinical and clinical results indicate that manipulation of \textit{CTLA-4} has considerable promise as a strategy for the immunotherapy of metastatic melanoma and a variety of other types of human cancer. However, not all patients responded well to the therapy and some patients developed severe autoimmune reactions. To identify genetic factors that might affect response to and toxicity of \textit{CTLA-4} antibody therapy, several \textit{CTLA-4} polymorphisms have been investigated (32). Patients with metastatic melanoma having the 49A allele showed better response to anti-\textit{CTLA-4} therapy than those having the 49G allele, which seems to be logical because the \textit{CTLA-4} 49A allele is correlated to enhanced inhibition of T-cell proliferation and activation. This result suggests that \textit{CTLA-4} genotype-haplotype may serve as genetic marker for personalized anti-\textit{CTLA-4} therapy although further studies are needed to verify this important finding.

\section*{Conclusion}

The \textit{CTLA-4} gene contains several SNPs that may influence gene expression, cause amino acid substitution, and alter mRNA splicing, which may manipulate T-cell activation and, eventually, modulate host immune status. Although functional alterations resulting from \textit{CTLA-4} polymorphisms seem to be subtle, the biological outcomes are significant. These genetic variations have been shown to be associated with autoimmune diseases and cancer in the opposite direction, and might also render interindividual differences in response to immunotherapy targeting this molecule. These findings indicate an important role of a dialectical nature of T-cell immune dysregulation in human disorders, such as autoimmune disease and cancer.

\section*{Disclosure of Potential Conflicts of Interest}

No potential conflicts of interest were disclosed.

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\section*{References}
