THE ROLE OF SINGLE NUCLEOTIDE POLYMORPHISMS OF CYTOKINE GENES IN VIRAL INFECTIONS

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Abstract - Gene polymorphisms result from evolutionary processes representing mutations that survive in the population with a frequency higher than 1%. The most investigated type of gene polymorphisms are single nucleotide polymorphisms (SNPs). The SNPs of IL-12B (rs 3212227) A/C among a population of kidney graft CMV-seropositive recipients have an impact on a clinical events in cytomegalovirus (CMV) disease. Constitutive -308 G/A TNF-α polymorphism (rs1800629) is related to the susceptibility of HR-HPV-associated cervical dysplasia and cancer. SNP located 3 kb upstream of the IL-28B gene (rs12979860) seems to be the strongest host genetic predictor of sustained virologic response (SVR) in hepatitis C genotype 1 patients. It is very important to identify viral and host genetic markers that may facilitate the risk of developing viral disease or some viral-associated cancers. In addition, these markers could be useful in the choice of effective treatments and preventive strategies against virally induced infection.

Key words: SNP, IL-12B, TNF-α, IL-28B, CMV, kidney recipients, HPV, cervical cancer, hepatitis C, SVR

Gene polymorphisms are the result of evolutionary processes representing mutations that survive in the population with a frequency higher than 1% (Attia et al., 2009). Within the human genome DNA polymorphisms occur every couple of hundred base pairs. The most investigated type of gene polymorphisms are single nucleotide polymorphisms (SNPs) represented by substitution, insertion or deletion of a single DNA base. The functionality of each SNP depends on its position in the gene, the most relevant ones being those in regulatory regions of the gene. Polymorphisms located in 5’ promoter/enhancer of the gene can control the level of gene transcription, exon polymorphisms often change the DNA coding sequence, polymorphisms in introns may affect differential RNA splicing, while polymorphisms in the 3’ UTR region can influence mRNA stability. There are an estimated 1.42 million SNPs in the human genome. Because of this, a reliable nomenclature with a reference sequence (rs) number for each SNP is required (Goldstein, 2009).

Recently, in order to define useful disease biomarkers, many immunogenetic and pharmacogenetic investigations have been performed worldwide. Biomarkers are biological characteristics that can be detected and measured, such as genes, gene products and various other molecules. It is essential to distinguish between disease-related and drug-related biomarkers. Disease-related biomarkers can be diagnostic biomarkers, if a disease already exists, or can suggest how a disease may develop in an individual
case (prognostic biomarker). Drug-related biomarkers indicate whether a drug will be effective in a certain patient and how the patient’s body will process it. Genomic biomarkers have an important place in the development of novel clinical and therapeutic approaches in patients with viral diseases, especially those that are on immunosuppressive therapy (Hirschhorn et al., 2005).

Recently, many reports in the literature have described the role of certain cytokine gene polymorphisms in the development of viral diseases, efficacy of antiviral therapy and even in the risk of developing tumors of viral origin.

**Association between a polymorphism in the IL-12B gene and cytomegalovirus reactivation in kidney transplant recipients**

Cytomegalovirus (CMV) infection remains one of the most frequent infection disease after renal transplantation, with an incidence in the kidney transplant population estimated to be between 8 and 32% (Hartmann et al., 2006). This herpes virus infects mostly adults and remains latent throughout life. CMV infection in transplant recipients is directly associated with a significant rate of morbidity and its indirect effects on transplant recipients have been increasingly recognized during recent year (Brennan, 2001). After transplantation, a number of donor-derived proteins are recognized as foreign antigens by the recipient’s immune system, resulting in acute rejection whereby grafted cells are rapidly and specifically eliminated by the recipient immune system. Immunosuppressive drugs are used to prevent this reaction, but at the same time, these treatments are generally associated with an increased risk of many infections. In response to these infections, the host usually mobilizes both innate and adaptive immunity to ensure appropriate control of the infection. Innate immunity plays a crucial role in limiting early viral replication through the activation of natural killer (NK) cells and interferon-γ (IFN-γ) production (Segedal et al., 2004). Specific immunity is mainly mediated by T lymphocytes, CD8+ T cells being primarily responsible for the viral clearance in peripheral organs (Lucin et al., 1992; Halary et al., 2005). Gamma delta T cells also participate in the early immune response to CMV, possibly through IFN-γ synthesis (Lafarge et al., 2001).

The T cell-mediated immune response is regulated through an intricate network of molecular signals, including interleukin-12 (IL-12) which directs T cell response towards Th1 differentiation and stimulates IFN-γ production in T and NK cells, acting as a major player in the balance between Th1- and Th2-type immune responses. IL-12p70, mainly produced by macrophages and dendritic cells (DC) after encountering the antigens, is composed of two subunits: p35 and p40 encoded by *IL-12A* and *IL-12B* genes located on two different chromosomes. An SNP in the 3’ untranslated region (3’UTR) of *IL-12B* (rs 3212227) is represented by A to C substitution at position +1188 in the mRNA, reducing the stability of the p40 mRNA, which results in the decrease of IL-12 p40 production by stimulated peripheral blood monocyte cells (PBMCs) (Stanilova and Miteva, 2005). This SNP appears to be clinically relevant in susceptibility to multiple sclerosis and psoriasis vulgaris, but in recent years it has also been described as a risk factor for the occurrence of CMV infection after kidney transplantation. Distribution of the genotypes of this IL-12B 3’UTR polymorphism in kidney-graft CMV-seropositive patients is reported to be 60% for AA genotype, 36% are AC heterozygotes, while CC genotype is present in 4% patients (Hoffmann et al., 2008). This distribution is in Hardy-Weinberg equilibrium, which indicates that its genetic diversity was stable. The C allele frequency is 22% and it is strongly associated with lower mRNA stability and lower production of the p40 protein (van Veen et al., 2001; Hoffman et al., 2009). Lower p40 expression in the C carriers might explain the pathogenic link between the IL-12B genotype and CMV replication. IL-12 p40 directly stimulates IFN-γ production by CD8+ T cells and both cytotoxic T cells and IFN-γ represent major mediators in the host defense against CMV. This suggests that the level of p40 expression may play an important role during CMV infection. IL-12B polymorphism (rs 3212227) in a population of kidney-graft CMV-seropositive recipients may have
an impact on the clinical course of the CMV disease. Therefore, the patients who are C allele carriers might greatly benefit from CMV prophylaxis, which should be administrated immediately after transplantation to all patients with a risk of CMV disease.

Association of TNF-α gene promoter polymorphism in HPV-associated cervical cancer

Another genetic biomarker related to the association with viral infection is tumor necrosis factor-α (TNF-α) and its gene that has been described in correlation with susceptibility to HPV-associated cervical dysplasia and cancer (Muñoz, 2000). Cervical cancer is the most common genital malignancy that has high morbidity and mortality among women around the world, especially in developing countries (Waggoner, 2003). Infection with high-risk human papillomavirus (HR-HPV) is an important factor in the development of this cancer. Persistent HR-HPV infection induces an inflammatory response and cytokines that play an important role in this process can influence the development of the disease (Gaiotti et al., 2000; Govan et al., 2006). A pronounced shift from a Th1 (pro-inflammatory) cytokine production to a Th2 (anti-inflammatory) cytokine production has been observed in contrast-induced necropathy (CIN) patients with extensive HPV infection (Jang et al., 2001), suggesting that the type of cytokine response in HPV infection may be influential in determining disease outcome. In addition, there is evidence that reduced Th1 cytokine levels and increased Th2 levels (Bais et al., 2005) are associated with poor prognosis in cervical cancer (Kirkpatrick et al., 2004).

TNF-α, a pro-inflammatory cytokine, plays a pivotal role in the pathogenesis of a variety of infectious and inflammatory diseases (Field, 2001). Previous reports indicated that TNF-α promotes the progression of cell cycle by increasing cyclin-dependent kinase activity and HPV16 E6/E7 mRNA expression in HPV-immortalized keratinocytes (Szlosarek et al., 2006). In addition, it has been shown that TNF-α enhances the transcription and stability of the epidermal growth factor receptor (EGFR), resulting in cell proliferation and tumorigenesis. Recently, it has been reported that the level of serum TNF-α in women with CIN was much higher than in healthy controls (Mocellin et al., 2005; Akkiz et al., 2009). In HPV-associated CIN, individual cytokine gene polymorphisms might potentially affect the disease process through several mechanisms, including the modulation of cytokine production in response to HPV. Single base polymorphism at the position -308 in the promoter of the TNF-α gene (rs1800629), represented by G to A substitution, is biologically very important. Its increased in vitro and in vivo transcription by 6- to 9-fold may affect susceptibility to cervical cancer (Stanczuk et al., 2003). This SNP can influence the expression of TNF-α, with the less common -308 A allele resulting in higher TNF-α production, and relates to a 2-fold increase in the risk of invasive cervical cancer. In contrast, the -308 G allele is linked to the reduced TNF-α production and low risk in HPV-associated cervical dysplasia (Kirkpatrick et al., 2004). Indeed, these observations presented the possibility that tumor development may be associated with the genetic predisposition of the host to produce higher levels of TNF-α (Deshpande et al., 2005). Numerous studies have investigated the association between the effect of TNF-α promoter region variations and cervical cancer, but the results have been contradictory (Calhoun et al., 2002). Notably, these findings are from various ethnic populations, indicating that the variation in TNF-α production may be influenced by the genetic make-up of ethnically diverse populations, contributing to the disparity in disease outcome (Ghaderi et al., 2001; Zuo et al., 2011).

Association of IL-28B polymorphism and response to therapy in hepatitis C patients

Hepatitis C infection is a major global public health problem. Among individuals infected with hepatitis C virus (HCV), approximately 80% develop chronic HCV infection. Chronic infection is the leading cause of liver cancer worldwide (Alavian, 2011). Successful treatment of chronic HCV infection results in a sustained virologic response (SVR), observed as undetectable post-treatment HCV RNA in the plac-
ma that greatly reduces the risk of cirrhosis and liver cancer. Current treatment for patients chronically infected with genotype 1 HCV requires 48 weeks of pegylated interferon (PEG IFN) plus Ribavirin (RBV) (Tanaka et al., 2009). The goal of this treatment is viral eradication, defined as the absence of virus 24 weeks after treatment completion, but it is usually achieved only in <50% of patients. Treatment of HCV infection with PEG IFN-α plus RBV, as a standard of care for the management of this disease, leads to the eradication of the virus in less than 60% of patients (Hoofnagle and Seeff, 2006).

Genome-wide association study (GWAS) found the SNP present 3 kb upstream of the \( \text{IL-28B} \) gene (rs12979860) to be the strongest host genetic predictor of SVR in HCV genotype 1 (Tanaka et al., 2009). \( \text{IL-28B} \) is located on chromosome 19 and it encodes a protein also known as IFN-λ3. In vitro studies have suggested that IFN-α induces the expression of interferon-λ genes (Maher et al., 2008) and that IFN-λ inhibits HCV replication through a pattern of signal transduction and regulation of interferon-stimulated genes, which is distinct from that of IFN-α. IFN-α and IFN-λ proteins both induce The Janus-activated kinase (JAK) and the signal transducer and activator of transcriptions (STAT) pathway upregulates several hundred interferon-stimulated genes. Through this mechanism IFN-α and IFN-λ suppress viral infections (Muir et al., 2010).

There are three \( \text{IL-28B} \) genotypes: CC, CT, and TT. Patients with the CC genotype have a stronger immune response to HCV infection than individuals with CT or TT genotypes (called non-CC genotypes). A stronger immune response most probably enables patients who have a CC genotype to clear the HCV within months of becoming infected without treatment. Patients with CC genotype are also 2 to 3 times more likely to be cured by PEG-IFN and RBV. Hence, two of the strongest SVR predictors are the virus-related amount of HCV in the bloodstream (HCV-viral load) and the HCV genotype (Thompson et al., 2010). Based on all the above-mentioned, genotyping of this polymorphism could aid clinical decision in defining the standard of care for these patients and providing an opportunity for clinicians to individualize treatment regimens for HCV patients.

In the light of all that has been discussed, it is very important to identify viral and host genetic markers that may facilitate the risk of developing viral disease or some viral-associated cancers. In addition, these markers could be useful in providing options for effective treatments and preventive strategies against virally induced infection.

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REFERENCES


