

Chapter 2

Pattern Recognition Receptors, Gene Polymorphisms, and Cancer: A Double-Edged Sword

2.1 Introduction

The novel approaches in healthcare move towards the model of “personalized medicine”. Advances in the healthcare service grow annually as well as their social relevance. Diagnostic tests and target therapy have become a part of our life. However, in spite of the neoteric improvements of the screening and treatment modalities, the prognosis of patients with many diseases including cancer remains poor. Thus, modern molecular biology and medicine are concerned on the developing of more and more new genomic markers that possess predictive, therapeutic, or prognostic significance. Several markers may evaluate predisposition of any person to one or another disease with a certain degree of accuracy based on the results of a simple blood test. The widespread application of these tests can reveal the risk groups in populations, and thereafter, the complex of preventive measures among the risk group subjects may be conducted. Moreover, above-mentioned genomic markers can be identified in the perinatal period, so the choice between “include” or “not to include” in the risk group on their basis can be made maximally early, and, consequently, the preventive measures can have the greatest efficacy. As a result, the integrative systems of predictive genomic markers, defined once, will allow to create the programs of cancer prevention based on them and will permit next generations to be informed and forewarned about their risks and predispositions to certain diseases.

Thereby, the discovery and development of predictive, therapeutic, or prognostic markers is the primary problem of biomedicine at the present time. However, the critical barrier for progress in this field is that it is not always easy to find an effective genomic marker that is exactly associated with a particular disease. One of the most widespread and important markers is the type of genomic markers called single nucleotide polymorphisms (SNPs). They represent a variation in the DNA sequence, when a single nucleotide differs between members of a biological species or paired chromosomes in an individual.

The finishing of Human Genome Project and widespread distribution of genotyping technologies have led to enormous number of studies devoted to association of inherited gene polymorphisms with various diseases. SNPs may

result in amino acid substitutions altering protein function or splicing, and they can also change structure of enhancer sequences during splicing [1] or affect mRNA stability [2]. SNPs may alter transcription factor binding motifs, changing the efficacy of enhancer or repressor elements [3], and can alter the structure of translation initiation codons that may lead to downregulation of wild-type transcript [4]. Gene polymorphisms located in leucine-rich repeats constituting ecto-domain of PRRs may affect the ability of receptor to bind pathogens they normally recognize [5], SNPs in transmembrane domain can lead to defects of intracellular receptor transport that do not allow to locate a receptor on the membrane [6], and, finally, polymorphisms in the internal domain may result in altered interaction with adaptor proteins or in disrupted dimerization. So, inherited SNPs of genes encoding PRRs may alter PRR expression and activity, modulating risk of various diseases and, possibly, influencing on various features of their progression. The same statement should be true for genes encoding proteins of PRR signaling pathways.

On the basis of plethora of fundamental and epidemiological studies carried out, it is possible to specify two fundamental mechanisms for modulation of risk of diseases by polymorphisms of genes encoding PRRs and proteins of PRR pathways. The first of them is impairment of immune response to certain pathogens (it can be bacteria, viruses, fungi, protozoan, and helminths) that increase risk of infection and promote its development along with further chronical persistence. The second mechanism is increase of production of proinflammatory cytokines after binding of the ligand (exogenous or endogenous) that create a condition of hazardous chronic inflammation.

Since PRRs mediate immune response induced by many immunoadjuvants [7, 8] and many of them regulate immune response against potentially carcinogenic infectious agents [9, 10] (*H. pylori* [11–14], EBV [15, 16], HPV [15, 16], HHV-8/KSHV [15, 16], *M. tuberculosis* [12–14, 17], *S. pneumoniae* [12–14, 18], enteropathogenic *E. coli* [12–14, 19], *S. flexneri* [12–14, 20], *S. typhimurium* [12–14, 21], *B. burgdorferi* [12–14, 22], *C. pneumoniae* [12–14], *C. trachomatis* [12–14, 23], *C. psittaci* [12–14], *Campylobacter jejuni* [12–14, 24] etc.), it seems to be possible to stimulate anti-tumor immunity through their enhanced activation [25, 26]. This hypothesis, originally developed for TLRs, should be also true for all PRRs as well [25, 26]. According to this suggestion, reinforced PRR activation may protect from infectious agents and prevent, inhibit, or block carcinogenesis whilst disrupted functioning of these PRRs may allow infectious agents or tumor cells to avoid recognition by immune system and, consequently, not to be eliminated [25, 26]. At the same time, such PRR activation may promote carcinogenesis, creating a proinflammatory microenvironment (via action of respective cytokines) that is favorable for tumor progression and chemoresistance development [27]. It may also result in immunosuppression caused by chronic inflammation [25]. Chronic inflammation may promote the development of cervical, endometrial, ovarian, breast, prostate, testicular, nasopharyngeal, lung, esophageal, gastric, colorectal, liver, pancreatic, gallbladder, kidney, bladder, lymphatic malignancies, and feasibly several other cancer types [8, 28]. In this case, on the

contrary, lower PRR activity should minimize effects of chronic inflammation such as enhancement of cancer initiation and promotion/progression and, consequently, decrease probability of tumor development [27]. So, the situation resembles a double-edged sword. The ideal variant, possibly, is the “golden mean”—the balance between low and high PRR activity. This hypothesis, initially developed for PRRs [26], may also be successfully projected on PRR intracellular signaling pathways—if their elements are overexpressed/constantly activated, it may lead to similar consequences as enhanced PRR activation. On the other hand, if members of PRR pathways are underexpressed/inactivated/unable to do their work, it may result in the same effects that arise after decreased PRR activity, and the analogical “golden mean” in functioning of all genes encoding proteins constituting PRR signaling pathways will be the optimal variant.

We will describe the molecular mechanisms of action of TLR and NLR polymorphisms on several examples. For instance, nonsynonymous polymorphisms *TLR4*_896A/G (major allele A, minor allele G, wild-type homozygous genotype is almost always A/A, rs4986790) and *TLR4*_1196C/T (major allele C, minor allele T, wild-type homozygous genotype is almost always C/C, rs4986791) are located in the fourth exon, affect extracellular domain of TLR4 and cause adenine–guanine (A–G) and cytosine–thymine (C–T) transitions, respectively. This, in turn, leads to amino acid substitutions: glycine instead of aspartic acid at 299 position (Asp299Gly) and isoleucine instead of threonine at 399 position (Thr399Ile) [29]. These substitutions lead to alteration of the ligand-binding receptor site [30], reach substantial frequencies and are found in cosegregation in Caucasian populations [31–33]. This cosegregated state of TLR4 implies that four haplotypes are represented in the population: wt/wt, Asp299Gly/wt, Thr399Ile/wt, and Asp299Gly/Thr399Ile [29]. Crystallography of the TLR4/LPS/MD-2 complex reveals two highly-preserved TLR4 regions, involved in binding the LPS/MD-2 complex [34]. These regions are located in N-terminal and central receptor domains [34]. The crystallography reveals that Asp299Gly is not directly involved in MD-2 binding but the mutation is close to the TLR4/MD-2-binding area [34]. Although direct alteration of the LPS/MD-2 complex binding area does not occur, it is possible that Asp299Gly increases the rotational freedom of the peptide bond [30]. Wild-type TLR4 has a negatively charged area at position 299, which is lost in Asp299Gly [30]. So, the functional peculiarities of cells with Asp299Gly may be the result of increased rotation and charge changing that may modulate the interaction between TLR4 and LPS [29]. The Thr399Ile polymorphism conserves the branched side chain but increases the overall steric bulk in this region, possibly precluding ligand or cofactor docking [30]. The presence of both Asp299Gly and Thr399Ile may lead to the loss of docking that cannot be overcome by the remaining interactions, and it is proven by observation that the doubly mutated TLR4 molecule consistently responds more poorly to ligand stimulation than TLR4 molecules that express either the Asp299Gly or Thr399Ile mutation [30]. Arbour et al. [32], basing on immunohistochemical staining, suggested that the Asp299Gly/Thr399Ile mutant protein may not be as well expressed as wild-type TLR4 molecule. Nevertheless, Rallabhandi et al. proposed alternative explanation:

according to their paper, Asp299Gly/Thr399Ile double mutant alters an immunodominant epitope that, in turn, results in diminished detection of mutant TLR4 molecules [32]. Despite comparable TLR4 surface expression, carriers of Asp299Gly and Thr399Ile demonstrated reduced responsiveness to LPS, chlamydial Hsp 60 and respiratory syncytial virus fusion protein, and, moreover, the double mutant displayed the most significant hyporesponsiveness [32]. However, the precise molecular mechanisms of the diminished LPS responsiveness of individuals with Asp299Gly and Thr399Ile and accurate mechanism by which these polymorphisms mediate their synergistic effects are not clarified enough [32].

Concerning NLRs, there are three independent major *NOD2/CARD15* gene polymorphisms. Two of them are missense mutations (rs2066844, Arg702Trp and rs2066845, Gly908Arg) [35] and one is frameshift mutation resulting in a premature stop codon and, consequently, in synthesis of truncated protein (rs2066847, 3020insC → Leu1007 fs) [36]. All these three variants are located in C-terminal region and are defective in their ability to respond to bacterial MDP [37], to activate NF- κ B and, hence, to stimulate the production of proinflammatory mediators [38, 39], whereas the fourth ubiquitous *NOD2/CARD15* SNP (rs2066842, Pro268Ser) alone exhibits wild-type activity [37]. In addition, insertion allele of rs2066847 polymorphism may disrupt the association of receptor with cell surface membranes [40]. Ten other missense polymorphisms (rs104895493, Glu383Gly; rs104895462, Arg334Trp; rs104895461, Arg334Gln; rs104895460, Leu469Phe; rs104895472, His496Leu; rs104895473, Met513Thr; Hrs104895474, Thr605Pro; rs104895475, Asn670Lys; combination of rs104895476, Asp382Glu and rs104895438, Ala612Thr), on the contrary, lead to NF- κ B upregulation in response to MDP stimulation [41, 42].

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