Article

Immunoregulatory network and cancer-associated genes: molecular links and relevance to aging

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Abstract

Although different aspects of cancer immunity are a subject of intensive investigation, an integrative view on the possible molecular links between immunoregulators and cancer-associated genes has not yet been fully considered. In an attempt to get more insights on the problem, we analyzed these links from a network perspective. We showed that the immunoregulators could be organized into a miRNA-regulated PPI network—the immunoregulatory network. This network has numerous links with cancer, including (i) cancer-associated immunoregulators, (ii) direct and indirect protein-protein interactions (through the common protein partners), and (iii) common miRNAs. These links may largely determine the interactions between the host's immunity and cancer, supporting the possibility for co-expression and post-transcriptional co-regulation of immunoregulatory and cancer genes. In addition, the connection between immunoregulation and cancer may lie within the realm of cancer-predisposing conditions, such as chronic inflammation and fibroproliferative repair. A gradual, age-related deterioration of the integrity and functionality of the immunoregulatory network could contribute to impaired immunity and generation of cancer-predisposing conditions.

Keywords immunoregulators; cancer genes; protein-protein interactions (PPI); PPI network; micro RNAs (miRNAs); aging.

1 Introduction

The interactions between host's immune system and cancer is a field of intensive investigation (for reviews see: Dunn et al., 2002; Croci et al., 2007; Kim et al., 2007; Gregory and Pound, 2011; Ibrahim et al., 2011; Schreiber et al., 2011; Zhang, 2011a). Recognition and elimination of continuously arising transformed cells is an important host protection process aimed at inhibiting carcinogenesis and eventually maintaining regular cellular homeostasis. On the other hand, cancer cells evolved the mechanisms to escape the control from the immune system. Although it seems paradoxical, the immune and cancer cells often use the same "tools" aimed at ensuring their specific activities. Among these "tools" are immunoregulators which comprise a wide range of molecules including cytokines, chemokines, growth factors, adhesion molecules, Toll receptors, eicosanoids and others (reviewed by Clayton, 2010). As mentioned above, many of them are expressed not only by immune but also by tumor cells, resulting in complex and often opposite effects on host's immunity and tumor progression (Kim et al., 2007; Apte and Voronov, 2008). For example, the pro-inflammatory cytokines of IL-1

family are abundant at tumor sites, being produced by cells of the tumor microenvironment or by the tumor cells (Apte and Voronov, 2008). While IL-1α reduces tumorigenicity by inducing antitumor immunity, another IL-1 member, IL-1β, promotes tumor angiogenesis, growth and invasiveness and also suppresses immune responses in the host (Song et al., 2003; Elkabets et al., 2010).

Here we analyze for the first time the possible molecular links between immunoregulators and cancer-associated genes from a network perspective. Since the age-related changes in the immune system ("immunosenescence") are believed to contribute to increased incidence of cancer in the elderly (Pawelec et al., 2010), we also included this aspect in the analysis.

2 Methods

2.1 Data sources and analytic tools

The list of immunoregulatory genes was data mined from the scientific literature and manually curated. They include the genes whose products were reported to activate or suppress immune functions. Cancer-associated genes were collected from scientific literature and from publicly available databases as described previously (for details see: Budovsky et al., 2009; Tacutu et al., 2010, and references therein). They were selected based on (1) mutations associated with a higher frequency of cancer, (2) consistent up- or down-regulation of gene expression, shown to be important for cancer initiation or progression, and (3) gene polymorphisms associated with greater predisposition or susceptibility to cancer. Longevity-associated genes were taken from the Human Aging Genomic Resources—GenAge Database (de Magalhães et al., 2009; http://genomics.senescence.info/genes) and the genes involved in major age-related diseases (ARDs) and associated conditions (e.g., chronic inflammation) were taken from the NetAge database (Tacutu et al., 2010; http://netage-project.org).

Protein-protein interaction (PPI) data was extracted from the BioGRID database (Stark et al., 2011; http://thebiogrid.org; human interactome release 3.1.71). Micro RNAs (miRNAs) and their experimentally verified targets were retrieved from the TarBase database (Papadopoulos et al., 2009; http://diana.cslab.ece.ntua.gr/tarbase/). Annotations of miRNAs involved in cancer were taken from the Human MicroRNA Disease Database (Lu et al., 2008), and their classification as either oncogenic or tumor suppressor miRNAs was taken from Wang et al. (2010).

DAVID Bioinformatics Resources 6.7 (Dennis et al. 2003; Huang et al. 2009; http://david.abcc.ncifcrf.gov) was used for the GO (Ashburner et al., 2000) and KEGG (Kanehisa et al., 2010) pathway analysis. The miRNA-regulated PPI network was constructed using the YABNA software program (Tacutu et al., 2010) and the graphical output of the network was generated using Cytoscape 2.8.0 (Shannon et al. 2003; http://www.cytoscape.org/).

2.2 Statistical evaluation

Chi-test was used for evaluation of the difference between the observed and expected sizes of gene overlaps. Significantly enriched GO categories and KEGG pathways were computed with DAVID Bioinformatics Resources 6.7, using Bonferroni correction. P-values less than 0.05 were considered statistically significant.

3 Results and Discussion

3.1 Immunoregulatory network

Comprehensive data mining revealed 179 genes that have been identified as being important players in the regulation of immune responses (Online Suppl Table 1). Most of them (149 genes) were found in the human interactome. This is important for the analysis as immunoregulators exert their effects via interactions with

each other and with their interacting proteins. Together with their partners and miRNAs (Suppl Table 2), the immunoregulators eventually form a miRNA-regulated PPI network which comprises 650 nodes (Fig. 1).

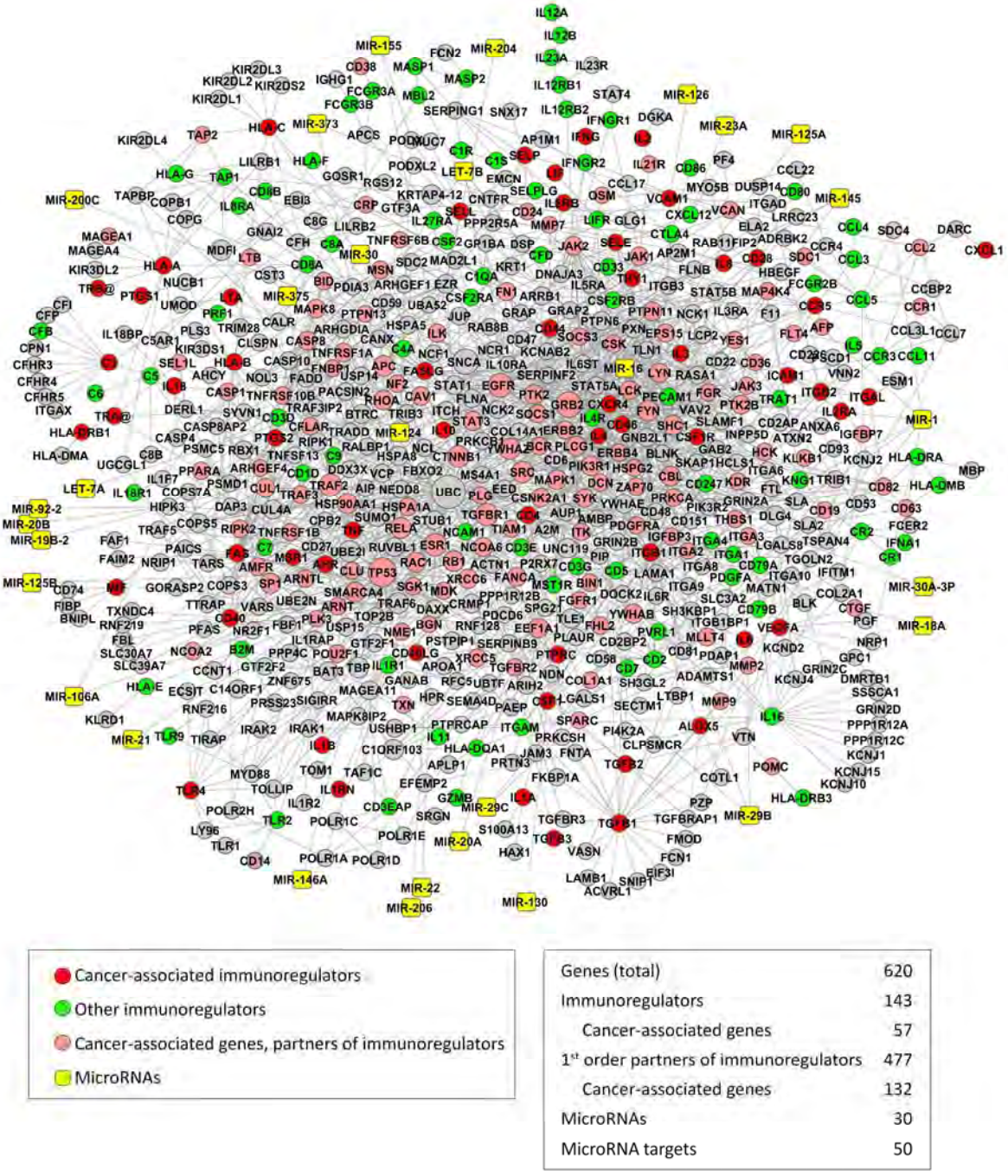

Fig. 1 The miRNA-regulated PPI immunoregulatory network. The network includes immunoregulators, their first-order partners, and miRNAs with experimentally validated targets. Node size is proportional to the number of PPIs for a given gene. In total, the network comprises 2096 connections.

The very possibility to organize the immunoregulators into an immunoregulatory network indicates that they function in a highly coordinated mode. Another important point is that the immunoregulatory network has a scale-free topology (Fig. 2), which is believed to ensure the network robustness, that is, the ability to resist random node attacks. Despite that, the immunoregulatory network may undergo age-related changes including loss of, or alterations in nodes (e.g, mutations, post-translational modifications) or their interactions and as a result, efficiency of the network decreases. This is in accordance with the numerous observations regarding the impact of aging on the immune system and *vice versa*, which have been reviewed in several papers, where implications for cancer were also addressed (Globerson and Effros, 2000; Kozak and Fraifeld, 2004; Shurin et al., 2007; Agarwal and Busse, 2010; Pawelec et al., 2010).

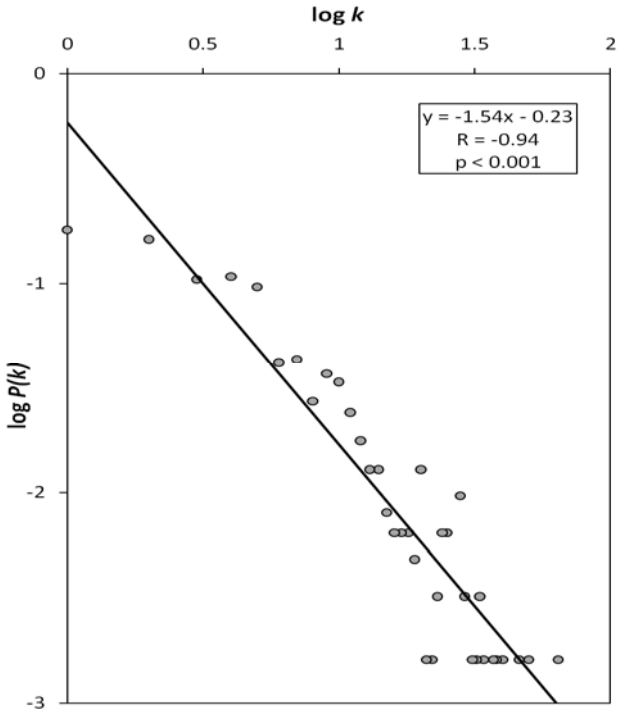

Fig. 2 The log–log plot and dependence of *P(k)* against *k*, illustrating a scale-free topology of the immunoregulatory network, which follows a power-law distribution of connectivity (Barabasi and Oltvai, 2004): $P(k) \sim k^{-\gamma}$, where *P(k)* is the probability that a selected node has exactly *k* connections (degrees) with other nodes; γ is the degree exponent which for most scale-free networks lies within an interval of $2 < \gamma < 3$. The smaller the γ value, the higher contribution of the hubs to the network connectivity.

3.2 Links between immunoregulatory network and cancer-associated genes

As shown in Fig. 3, at least 61 immunoregulators were also identified as cancer-associated genes (cancer-associated immunoregulators). Of them, 57 are found in the immunoregulatory network. The existence of many common genes is the first and most obvious evidence for the connections between the immunoregulatory and cancer-associated genes, suggesting that the same stimuli may induce similar gene responses in the immune and cancer cells. The induction or suppression of a given gene could, however, result in different or even opposite cellular responses. For example, IL-1-induced upregulation of cyclooxygenase 2 (COX-2), with subsequent elevation of PGE_2, acts as a feedback inhibitor of cellular immune responses (Goodwin and Ceuppens, 1983), whereas in tumors, PGE_2 elicits multiple oncogenic signals to promote carcinogenesis (Wang and Dubois, 2010; Wu et al., 2010). Of note, the mitogen-stimulated PGE_2 production is significantly

increased in old age, being in part responsible for a reduced proliferative capacity of the immune cells (Fraifeld et al., 1995).

Further strengthening the links between immunoregulators and cancer is that a high portion of immunoregulatory proteins (n = 53) directly interact with proteins involved in cancer (n = 132) and much more may interact via their common protein partners (n = 313) (Fig. 3).

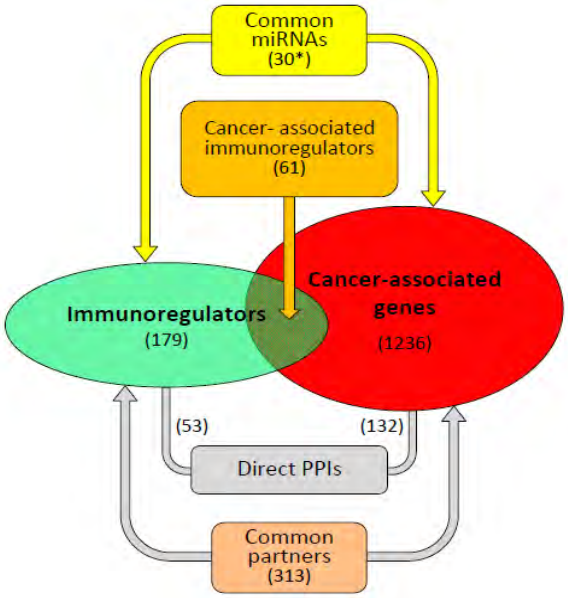

Fig. 3 Schematic representation of molecular links between immunoregulators and cancer-associated genes. *Of the 30 miRNAs which have targets in the immunoregulatory network, 27 miRNAs have also targets among cancer-associated genes (9 miRNAs target immunoregulators and 18 miRNAs target their partners).

In total, almost one third of the genes (n = 189) in the immunoregulatory network overlap with cancer-associated genes (Fig. 1). This value is twofold higher than expected by chance ($p < E{-}25$).

Many genes from the immunoregulatory network are validated targets of miRNAs (Fig. 1, Online Suppl Table 2). Almost all of these miRNAs may have a pleiotropic effect by targeting more than one gene, including cancer-associated genes (n = 99). As such, the common miRNAs could be another line of possible co-regulation of immune responses, cancer development and interaction between them. Important to stress that among the targets of the above miRNAs are also longevity-associated genes (Wolfson et al., 2008; Tacutu et al., 2010). Moreover, the vast majority of the miRNA targets, in addition to cancer, have been reported to be involved in at least one major ARD or aging-associated condition, such as chronic inflammation (Online Suppl Table 3). Thus, the common miRNAs could actually represent one of the bridges connecting immunoregulation, cancer, and aging/longevity.

3.3 GO function and KEGG pathway analysis

To get further insight into the links between the immunoregulatory network and cancer, we undertook a functional analysis of the common genes, that is, cancer-associated genes found in the immunoregulatory network (n=189 genes). The analysis showed more than 350 enriched GO – biological process categories ($p < 0.05$, with Bonferroni correction; Online Suppl Table 4). The most enriched categories were related to regulation of cell proliferation, cell communication, response to wounding, programmed cell death, inflammatory response and as expected, to the various aspects of immune responses.

Enrichment with genes related to regulation of cell proliferation is quite obvious as this process is critical for both immune and cancer cells. Indeed, proliferation of the immune cells ensures a massive attack against cancer cells, while continuous proliferation of cancer cells ensures tumor growth. An immune (inflammatory) component is also essential for the initial phases of wound healing (Gurtner et al., 2008). On the other hand, this fundamental biological process and cancer have much in common and Schäfer and Werner (2008) even considered "cancer as an overhealing wound". With regard to this, it should be noted that the frequency of fibroproliferative conditions dramatically increases with advanced age and this occurs in parallel with an increased incidence of cancer (Cutler and Mattson, 2006). Another important point is a significant overrepresentation of chronic inflammation genes in the immunoregulatory network. The overlap comprises 64 genes, six times higher than expected by chance ($p < E{-}25$). Of these 64 genes, 47 are also cancer-associated. This coincides well with the notion that chronic inflammation is strongly associated with promotion of malignancy and tumor progression (Elkabets et al., 2010). Moreover, strong links between cancer-associated immunoregulators and chronic inflammation are in favor of the widely accepted assumption that chronic inflammation may underpin the aging process, cancer and other ARDs (Chung et al., 2009; Fulop et al., 2010; Rodier and Campisi, 2011). Recently, this assumption gained further support from the discovery of the phenomenon of Senescence-Associated Secretory Phenotype (SASP) (Coppé et al., 2008; Campisi, 2011). It was shown that senescent cells secrete a wide plethora of pro-inflammatory and pro-cancer molecules (Davalos et al., 2010; Freund et al., 2010). These cells accumulate in aging and are found in tumor microenvironment (fibroblasts, endothelial cells). Nineteen SASP genes are also immunoregulators and almost 40% ($n = 28$ genes) of all currently reported SASP molecules are found in the immunoregulatory network.

The KEGG pathway analysis revealed 42 enriched pathways ($p < 0.05$, with Bonferroni correction), including also several pathways related to various cancers (Online Suppl Table 5). Remarkably, among the topmost enriched pathways are focal adhesion and adherens junction involved in cell-cell and cell-extracellular matrix interactions. As we previously showed, these pathways are considerably overrepresented in major human ARDs including cancer (Wolfson et al., 2009). Thus, any dysfunction in these pathways may have far-reaching consequences not only for immunity and cancer but also for the aging process and associated pathology.

4 Concluding Remarks

It has become increasingly clear that biological systems function as complex networks (Barabasi and Oltvai, 2004; Budovsky et al., 2007; Tacutu et al., 2010; Ibrahim et al., 2011; Zhang, 2011a,b and references therein). The holistic, network-based approach opens new possibilities for understanding the functional organization of processes which include a great number of genes. Here we demonstrate that the immunoregulators, an important part of the immune system, can be organized into a miRNA-regulated PPI network—the immunoregulatory network, highlighting their cooperative mode of action. This network has numerous links with cancer, including (i) cancer-associated immunoregulators, (ii) direct and indirect PPIs (through the common protein partners), and (iii) common miRNAs. These links may largely determine the interactions between the host's immunity and cancer, supporting the possibility for co-expression and post-transcriptional co-regulation of immunoregulatory and cancer genes.

Another line of connections between immunoregulation and cancer lies within the realm of cancer-predisposing conditions, such as chronic inflammation and fibroproliferative repair, in which immune responses play an essential role.

Today it is widely accepted that aging is the major risk factor for the development of ARDs including cancer (Cutler and Mattson, 2006). Even more convincing is that exceptional longevity or longevity-promoting

interventions usually lead to the delay of or escape from cancer and also to better preservation of the immune functions (Spaulding et al., 1997; Dixit, 2008; Blagosklonny, 2008; Budovsky et al., 2009; Agarwal and Busse, 2010; Derhovanessian et al., 2010). Age-related changes in the integrity and functionality of the immunoregulatory network could contribute to impaired immunity and generation of ARD-predisposing conditions. The proper maintenance of the network could therefore be the main strategic goal for tackling immunoregulatory disorders and age-associated pathology.

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