Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Tumor-induced perturbations of cytokines and immune cell networks ☆

Brett Burkholder [a], Ren-Yu Huang [b], Rob Burgess [a], Shuhong Luo [a,b], Valerie Sloane Jones [a], Wenji Zhang [b], Zhi-Qiang Lv [b], Chang-Yu Gao [b], Bao-Ling Wang [b], Yu-Ming Zhang [b], Ruo-Pan Huang [a,b,c,*]

[a] RayBiotech, Inc., 3607 Parkway Lane, Suite 100, Norcross, GA 30092, USA
[b] RayBiotech, Inc., Guangzhou 510600, China
[c] South China Biochip Research Center, Guangzhou 510630, China

ARTICLE INFO

Article history:
Received 2 November 2013
Received in revised form 3 January 2014
Accepted 4 January 2014
Available online xxxx

Keywords:
Cytokine
Cancer
Immunosurveillance
Immunosuppression

ABSTRACT

Until recently, the intrinsically high level of cross-talk between immune cells, the complexity of immune cell development, and the pleiotropic nature of cytokine signaling have hampered progress in understanding the mechanisms of immunosuppression by which tumor cells circumvent native and adaptive immune responses. One technology that has helped to shed light on this complex signaling network is the cytokine antibody array, which facilitates simultaneous screening of dozens to hundreds of secreted signal proteins in complex biological samples. The combined applications of traditional methods of molecular and cell biology with the high-content, high-throughput screening capabilities of cytokine antibody arrays and other multiplexed immunoassays have revealed a complex mechanism that involves multiple cytokine signals contributed not just by tumor cells but by stromal cells and a wide spectrum of immune cell types. This review will summarize the interactions among cancerous and immune cell types, as well as the key cytokine signals that are required for tumors to survive immunoediting in a dormant state or to grow and spread by escaping it. Additionally, it will present examples of how probing secreted cell–cell signal networks in the tumor microenvironment (TME) with cytokine screens have contributed to our current understanding of these processes and discuss the implications of this understanding to antitumor therapies.

Contents

Abbreviations: APC(s), antigen presentation cell(s); ARG1, arginase-1; Breg(s), regulatory B (cells); CAF(s), cancer-associated fibroblast(s); CD, cluster of differentiation protein; CD40L, cluster of differentiation protein 40 ligand; C/EBP, CAAT/enhancer binding protein; CCL, C–C motif (chemokine) ligand family member; CCR, C–C motif (chemokine) receptor family member; COX, cyclooxygenase; CTL(s), cytotoxic T lymphocyte(s); CTLA-4, cytotoxic T lymphocyte-associated protein-4; CXCL, C-X-C motif (chemokine) ligand family member; CXCR, C-X-C motif (chemokine) receptor family member; CSF, colony stimulating factor; DC(s), dendritic cell(s); ECM, extracellular matrix; EGF, epidermal growth factor; ELR+, contains amino-acid sequence "glutamate–leucine–arginine"; ENA-78, neutrophil activating peptide 78 (CXCL5); FasL, Fas ligand (TNFSF6); G-CSF, granulocyte colony stimulating factor (CSF3); GM-CSF, granulocyte-macrophage colony stimulating factor (CSF2); Gr-1, granulocytic myeloid marker protein; GRO, growth related oncogene α (KC/CXCL1), β (MIP-2/CXCL2) and/or γ (CXCL3); HIF-1α, hypoxia-induced factor-1α; HNSCC, head and neck squamous cell carcinoma; IDO, indolamine 2,3-deoxygenase; IFN, interferon; IgA, immunoglobulin A; IGF-1, insulin-like growth factor 1; IκB, inhibitor of kappa B; IL, Interleukin; IP-10, IFN-γ-induced protein 10 (CXCL10); IRF, interferon regulatory factor; LGALS, galectin (lecithin, galactoside-binding, soluble protein); KC, keratinocyte chemoattractant (mouse CXCL1); M1, type 1 macrophage; M2, type 2 macrophage; M-CSF, macrophage colony stimulating factor (CSF1); MCP-1, monocyte chemoattractant protein-1 (CCL2); MDSC(s), myeloid-derived suppressor cell(s); MHC, major histocompatibility complex; MIG, monokine induced by IFN-γ protein (CXCL9); MMP, matrix metalloproteinase; MPO, myeloperoxidase; MyD88, myeloid differentiation primary response gene 88; N1, type 1 neutrophil; N2, type 2 neutrophil; NF-κB, nuclear factor kappa B; NK(s), natural killer (cells); NK-T(s), natural killer T (cells); NO, nitric oxide; NOS2, nitric oxide synthase 2; PD-1, programmed death receptor 1; PD-L1, programmed death receptor 1 receptor; PGE_2, prostaglandin E_2; RNOS, reactive nitrogen oxide species; ROS, reactive oxygen species; SDF-1, stromal cell-derived factor 1 (CXCL12); SMAD, homolog of *Drosophila* "mothers against decapentaplegic" protein; TAAs, tumor-associated antigens; TCR, T-cell receptor; TGF, transforming growth factor; TGFBR2, type II TGF-β receptor protein; Th0, immature T helper cell precursor; Th1, type 1 T-helper cell; Th2, type 2 T-helper cell; Th17, type 17 T-helper cell; TIMP, tissue inhibitor of metalloproteinases; TME, tumor microenvironment; TNF, tumor necrosis factor; TNFRSF, TNF receptor superfamily member; TNFSF, TNF superfamily member (ligand); Treg(s), regulatory T (cells); TAM(s), tumor-associated macrophage(s); TAN(s), tumor-associated neutrophil(s); VEGF, vascular endothelial growth factor

* Corresponding author at: RayBiotech Inc, 3607 Parkway Lane, Suite, Norcross, GA 30092, USA.
E-mail address: rhuang@raybiotech.com (R.-P. Huang).

0304-419X/$ – see front matter © 2014 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbcan.2014.01.004

1. Introduction

Tumors are not homogenous population of cancerous cells; instead, tumors are heterogeneous groups of cells from diverse origins, such as stem cells, stromal cells, endothelial cells and a wide range of immune cells [1]. These heterogeneous populations secrete multiple signals that, individually and collectively, may promote or hinder any or all of the hallmarks of cancer required for tumor growth, development, and progression, as well as the enabling characteristics of genomic instability and tumor-promoting inflammation [2]. The immune system is constantly patrolling the body for foreign invaders and aberrant cells to destroy, a process commonly referred to as immunosurveillance [3]. Thus, to remain viable and to continue to grow and thrive, tumors must not just blunt the antitumor immune response in its microenvironment, the growing tumor must maintain other hallmarks of cancer, such as avoiding apoptosis and maintaining self-sufficiency of growth signals, inflammation, and angiogenesis [4].

Tipping the balance of immunosurveillance from tumor elimination to tumor promotion appears to be a complex process that spans multiple signal pathways that can be influenced by cytokine expression from tumor cells [5], immune cells [6], and other non-cancerous cell types, such as epithelial cells or cancer-associated fibroblasts (CAFs) [7], in the surrounding tissue. Thus, net cytokine signal inputs from multiple cell types in the tumor microenvironment (TME) can tip the overall balance in immunoediting from tumor-promoting to tumor-suppressing, or vice versa. As we described in a previous review, cytokine biology is quite complex [8]. How, then, does one go about decoding such a complex network of multiple signals (some of which may be additive, antagonistic, or synergistic) that may originate from multiple potential sources and pleiotropic effects on the heterogeneous cell types in the TME?

A proteomic technique that has shown great promise in detecting key cytokines in cancers and in decoding tumor-related cytokine signal networks is the multiplexed immunoassay [9–13]. The two major types of multiplexed immunoassays are antibody arrays (wherein capture antibodies are printed on a planar solid support, such as membranes, glass slides, or microtiter plates) and bead-based assays, in which capture antibodies are attached to fluorescently-tagged beads. The capabilities, challenges, advantages, and disadvantages associated with these various multiplexed immunoassay technologies—compared with one another and with more traditional approaches to proteomics and genomics—have been well documented in the literature [8,13–17] and are beyond the scope of this review.

However, the salient aspects of these methods that make them useful are the sensitivity and specificity of immunoassays and the ability to detect multiple proteins as once. Low-density bead-based assays and antibody arrays may detect a dozen or so targets simultaneously, but high-density (high-content) antibody arrays can potentially screen for hundreds of proteins in as little as 20 μl of sample. Thus, targeted screens for known inflammatory, angiogenic, apoptotic, or growth factors can help identify key biomarkers related to cancer hallmarks from among many potential candidates. High-density screens that allow for detection of cytokines with diverse functions may reveal crucial but unexpected expression of key factors by one or more cell types. Moreover, the heterogeneous nature of tumors lends itself naturally to analysis of the collective secretion profiles within tumor lysates or to dissect secretion profiles of single cell types (cancerous or non-cancerous) or co-cultures in vitro to discover which cells are contributing these critical signals and which cells are receiving them. After key factors are identified by these cytokine screens, more traditional methods of cell biology, molecular biology, and genetics can be used to complement these results, confirming and further dissecting and defining the complex network of secreted cell–cell signals and intracellular signaling pathways that contribute to the biological process being studied. Thus, antibody arrays are extremely powerful tools for the identification of cancer-specific biomarkers either secreted by cells in the local microenvironment or by the cancer cells themselves.

The balance of this review will summarize much of what is known of the cellular and secreted signaling networks that constitute the native

Please cite this article as: B. Burkholder, et al., Tumor-induced perturbations of cytokines and immune cell networks, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbcan.2014.01.004