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Thirty years after “interferon-β_2”: interleukin-6 at the host-tumor interface

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My laboratory stumbled on to “interferon-β_2” (IFN-β_2) in 1980 and subsequently cloned and assigned the human gene to chromosome 7. By 1988 this cytokine, also independently discovered by several other investigators, was dubbed “interleukin-6” (IL-6). Already in 1988-1989 we discovered that IL-6 was an almost invariant presence at the host-tumor interface in a variety of human solid tumors with both the tumor cells and stromal elements showing strong-to-moderate IL-6 immunoreactivity. The early studies also showed that circulating IL-6 was commonly increased in cancer patients and that glucocorticoids and estradiol-17β inhibited the IL-6 promoter. Today, the contributions of IL-6/STAT3 signaling have emerged as central to the interplay between infection and cancer, in promoting cancer metastases (e.g. breast, colon, liver) and in explaining gender- and obesity-related bias in cancer incidence (e.g. of liver cancer – less in women compared to males but high in obesity). Moreover, the well-known aging-related increase in cancer incidence is also now increasingly explained in terms of a “senescent secretory phenotype” which includes the increased production of IL-6 by tumor, stromal and infiltrating cells. Indeed, our early data (1991-1993) showing that cancer-derived mutants of p53 upregulated the IL-6 promoter, in contrast to wild-type (wt) p53 and wt Rb which inhibited, already pointed to a mechanism for dysregulated autocrine production of IL-6 by cancer cells – a mechanism that has since been extended by others. Additionally, it was shown already in 1989-1994 by Tamm and colleagues that IL-6 affected the “social” behavior of breast cancer cells – increased motility, cell-cell and cell-substrate dyshesion and epithelial-to-mesenchymal transformation. That these effects underlie the ability of paracrine or autocrine IL-6 to enhance the invasiveness and metastasis ability of tumor cells is now evident in animal models. In addition to the “canonical”, i.e. transcriptional functions of IL-6-activated STAT3, recent observations on the IL-6-induced targeting of activated Tyr-phosphorylated STAT3 to cytoplasmic sequestering endosomes and the involvement of non-Tyr-phosphorylated STAT3 in regulating microtubule dynamics, Ras-mediated cell transformation and tumorigenicity, and mitochondrial respiration highlight novel “non-canonical” functions of STAT3 in the cytoplasm of cancer cells.

Key words: interleukin-6, breast cancer, epithelial to mesenchymal transformation (EMT), cancer cell motility, metastasis, p53, STAT3.

Introduction

This year marks the 30th anniversary of when my laboratory stumbled upon “interferon-β_2” as a cytokine with observable antiviral activity derived from an mRNA of length 1.3 kb induced (“superinduced”) in human diploid fibroblasts by a combination of poly(I).poly(C) and cycloheximide [1]. This 1.3 kb mRNA did not cross-hybridize with a cDNA probe corresponding to the coding region of the then already cloned interferon-β (colloquially then called IFN-β_1); moreover the latter had an mRNA length of 0.9 kb [1]. Eventually cloning of the cDNA of interferon-β_2, elucidation of its deduced amino acid sequence, cloning its gene and the assignment of this gene to human chromosome 7 followed [2-4 and citations therein]. It then became clear that several investigators in the 1980s had independently discovered this cytokine in the guise of various other activities (e.g. B-cell stimulatory factor 2, hybridoma/plasmacytoma growth factor, hepatocyte stimulating factor, 26 kDa protein, interleukin-HP1 and monocyte granulocyte inducer type 2) [4, 5]. In December 1988 at a meeting on this cytokine in New York City, under the auspices of the New York Academy of Sciences, agreement was reached among all those who had independently discovered this cytokine to call it “interleukin-6” (IL-6) [5]. Parenthetically, the antiviral activity of IL-6 has now been best observed in *STAT1*-null cells [6]. By 1994 several additional cytokines such as leukemia inhibitory factor (LIF), interleukin-1 (IL-11), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), and cardiotrophin-1 (CT-1) that use the same gp130 signal transducing chain in their respective plasma membrane receptors for ligand-driven signaling to the cell interior were collectively grouped as IL-6-type cytokines [7]. Since then additional cytokines belonging to this group, i.e. those that use gp130 or a gp130-like (GPL) protein for signal transduction, have been discovered [e.g. new neurotrophin-1 (NNT-1) and IL-31] [8-10].

We realized already in 1988-1989 that IL-6 had an almost invariant presence at the host-tumor interface [11, 12]. A PubMed search in March 2010 using the terms “IL-6” and “cancer” returned over 9600 citations. Indeed there has been a veritable explosion of interest today in the involvement of dysregulated autocrine and paracrine IL-6 in affecting the biology of solid tumors, in mediating the gender bias in the incidence of liver cancer, in the relationships between cancer and inflammation, between cancer and obesity, between cancer and aging, and in enhancing cancer metastasis [12-20]. We realize that investigators new to the area of IL-6 and cancer may not be sufficiently aware of the early work that was already carried out in the years soon after 1988-89 [21]. Thus, in this review we shall briefly focus on examining aspects of the earlier work pertaining to the biology of IL-6 at the host-tumor interface and relate that early work to the current explosion in interest and activity in this area (Figure 1). Each of the issues summarized in Figure 1 – dysregulated production, chaperoned dissemination, alterations in signaling pathways and effects on the social behavior of cancer cells – is affected by considerations of “personalized oncology” in that the biology of IL-6 in cancer is affected by modifier gene mutations (such as in p53) which affect the production and action of this cytokine.

1) Dysregulated IL-6 production in a "personalized" manner – tumor cells, stromal cells, infiltrating "guest" cells; effects of p53 mutations; autocrine and paracrine production

2) IL-6 secretion – local and vascular dissemination – regulated bioavailability – chaperoned complexes – "personalized" properties in different cancer patients

3) Alterations in IL-6 signaling pathways in cancer cells – "personalized" effects dependent on mutations in modifier genes (e.g. p53 and caveolin-1 mutations affecting signaling via C/EBP and STAT3 signaling); paracrine/autocrine activation of PY-STAT3

4) Effects on the "social" behavior of cancer cells (focus on solid tumors) – cancer cell motility, invasiveness, cell-cell and cell-substrate dyshesion, epithelial-to-mesenchymal transformation (breast, colon, prostate).

Fig. 1. IL-6 is a common presence at the host-tumor interface: four aspects of the biological considerations

Dysregulated IL-6 production

In 1989 we reported, using immunohistochemistry, the presence of IL-6 in neoplastic cell elements and stromal tissue of primary carcinomas of mammary, colonic, ovarian and endometrial origin as well as in adenocarcinomatous metastases to lymph nodes [11]. Increased levels of circulating IL-6 were reported by others and by us in patients with various leukemias and solid tumors and cancers [12, 22, 23]. The concept of dysregulated production of IL-6 in the context of tumors [11, 12], either in an autocrine manner or in a paracrine manner, by infiltrating cells such as macrophages or stromal cells, has gained currency in recent years [13-20]. As examples, Karin and colleagues have highlighted IL-6 production by macrophages invading liver cancer in a murine model leading to increased tumorigenesis and metastasis [14] and also in models of colon cancer [reviewed in 13]. Others have highlighted stroma-derived IL-6 contributing to increased aggressiveness of breast cancer [18-20].

In 1988-1990 we extensively dissected the regulatory elements in the human IL-6 promoter [24, 25] and showed that it was markedly inhibited by glucocorticoids [26]. These observations were extended further to show that IL-6 production and promoter function were inhibited by estradiol-17β [27, 28]. These early observations become relevant in the present oncology context in that Karin and colleagues showed in 2007 that the increased incidence of liver cancer in males compared to females in a murine model (and by suggestion in humans) relates to the ability of estradiol-17β to inhibit IL-6 production by cancer-infiltrating macrophages induced to produce IL-6 by dead and necrotic cells through a MyD88-dependent pathway [14, 17]. Moreover, it is now known that single-nucleotide polymorphisms (SNPs) in the promoter region of the IL-6 gene can significantly affect IL-6 production in an individual-specific manner [29 and citations therein]. For the moment, though, studies of relationships between IL-6 promoter SNPs and cancer risk remain ambiguous [reviewed in 29].

At the molecular level we reported in 1991 that wild-type (wt) Rb and wt p53 but not cancer-derived p53 mutants inhibited the human IL-6 promoter [30]. This was extended to the observation that cancer-derived mutant p53 species enhanced the activity of IL-6 promoter constructs in a cell-type and p53-mutant dependent manner, especially by functional interactions with the C/EBPβ transcription factor [30-32]. Subsequently Kubicka *et al.* [33] showed the ability of wt p53 to inhibit and of various mutant p53 species to not affect C/EBPβ-driven promoters in liver cancer cells. Schneider-Merck *et al.* [34] confirmed the physical interaction between p53 and C/EBPβ. Importantly, Shi *et al.* [35] showed that IL-6 secretion *per se* is upregulated by mutant p53 species in prostate cancer cells to an extent that is specific for individual mutants, but not by wt p53. Thus, the suggestion in 1993 by us that mutations in p53 represent one molecular mechanism for the dysregulated upregulation of IL-6 production by cancer cells [31] has now been validated.

In a recent exciting development Campisi and colleagues have defined a "senescent-associated secretory phenotype" (SASP) characterized by increased secretion of IL-6 and IL-8 by normal fibroblasts and epithelial cells involving senescence associated with mutations in Ras and in p53 [19, 20, 36]. SASP factors, largely IL-6 and IL-8, induced an epithelial-mesenchyme transition and invasiveness – the hallmarks of malignancy – by paracrine mechanisms. Oncogenic ras expression and loss of functional p53 accelerated development of the SASP [36]. Thus, Campisi and colleagues ascribe the age-related development of cancer to increased dysregulated production of paracrine IL-6 and additional cytokines (such as IL-8) [19, 20, 36].

"Chaperoned" circulating IL-6 in cancer patients

It is now well established that the human circulation always contains substantial amounts of soluble IL-6 receptor and soluble gp130 [37]. Moreover, additional IL-6-binding proteins such as C-reactive protein, and IL-6 in circu-

lating complexes, are also typically found in the blood [38]. In melanoma patients subjected to immunotherapy we reported large quantities of IL-6 in complexes in plasma with varying biological activity and varying immunoreactivity depending upon which mAb-based ELISA was used [23, 39]. The existence of chaperoned IL-6, which then affects the bioavailability and bioactivity of IL-6 in the circulation, is an issue that has been inadequately considered as part of why different cancer patients might be affected by IL-6 differently. That a so-called “neutralizing” mAb to IL-6 can in fact enhance the biological activity of this cytokine in vivo has been demonstrated [40]; the IL-6/mAb complex likely forms a long-lived reservoir for the slow release and thus enhanced biological activity of this cytokine in vivo. Overall, the regulation of the bioavailability of circulating or local IL-6 is a research area that has been insufficiently investigated. It remains a puzzle how IL-6 becomes biologically available distant from its site of production in light of the observations that there is always a molar excess of the binding proteins sIL6R and sgp130 in blood [37-39].

“Personalized” differences in signaling pathways in cancer cells

With the realization that IL-6 signaling initiated at plasma membrane rafts progresses towards the cell interior along the endocytic pathway (“signaling endosome pathway”) [41-44] came the understanding that a number of proteins involved in these pathways (caveolin-1 is one example) can affect the strength and nature of this inward signal [41-44]. Thematically, endosomal signaling pathways are implicated in the traverse of activation signals through the cytoplasm to the nucleus elicited by a large number of growth factors and cytokines (reviewed in ref. 44). Proteins such as caveolin-1 (cav-1) and clathrin, which coat respective endocytic elements and adaptor molecules such as SARA, HRS, NF2, schwannomin, affect signaling in cancer cells (reviewed in ref. 44). Thus *cav-1* was also identified as a tumor suppressor gene in breast cancer [45]. In the case of IL-6 signaling, loss of cav-1 enhanced IL-6/gp130/STAT3 signaling in endothelial cells [46] while overexpression of cav-1 and the chaperone protein GRP58/ERp57 inhibited signaling [47].

Additionally, mutations in p53 affect the ability of IL-6 to elicit responses in cancer cells with wt p53 inhibiting and mutant p53 species enhancing such signaling both in terms of C/EBPβ and STAT3-elicited functions [31, 33, 48-50]. The discovery of a PY-STAT3 sequestering endosome compartment points to new functions of IL-6 in the cytoplasm [51, 52]. Finally, the involvement of non-Tyr-phosphorylated STAT3 in regulating microtubule dynamics, Ras-mediated cell transformation and tumorigenicity, and mitochondrial respiration highlight novel “non-canonical” functions of STAT3 in the cytoplasm of cancer cells [reviewed in 52, 53].

Effects of IL-6 on the “social” behavior of cancer cells

In a line of research between 1988 and 1994, Tamm and colleagues observed, primarily using time-lapse cinemicrophotography of breast cancer T47D and ZR-75-1 cell lines and subclones of ZR-75-1 typically extending over 9-10 days, that IL-6 caused the epithelial to fibroblastoid transformation of breast cancer cells [54-58, reviewed in 21]. Untreated T47D cells formed flat epithelioid colonies with tightly apposed cell-cell junctions while ZR-75-1 cells formed multilayered three-dimensional epithelioid colonies. IL-6 dispersed these colonies accompanied by “epithelioid to fibroblastoid” transformation [54-58]. Thus, IL-6 elicited a major change in cell phenotype which was characterized by a fibroblastoid morphology, enhanced motility, increased cell-cell separation, and decreased adherence type junctions (desmosomes and focal adhesions). The data identified IL-6 as a regulator of epithelial cell growth and of cell-cell association. IL-6-treated T47D cells showed loss of vinculin and desmoplakin I/II, decreased F-actin stress fibers, perinuclear retraction of cytokeratin filaments and diminished intercellular keratin filament connections and cytokeratins [54-58].

In 1990, I commented “The IL-6-induced phenotypic change, which is reversible, is of interest because (i) this change resembles that which certain epithelial cells undergo in early embryogenesis as they detach from the epithelium, move, and become mesenchymal in character, and (ii) it raises the question whether such changes may play a role in the invasiveness and metastasizing ability of tumor cells” (pg. 186 in ref. 12; also see ref. 21). The effect of IL-6 on the social behavior of breast cancer cells has now been confirmed in 3D culture experiments and in murine models [59, 60]. Moreover, these effects have also been observed in colon and prostate cancer [61, 62].

The emphasis today on the interplay between infection and cancer, with IL-6 now identified as a major participant in that interplay [13-18 and citations therein] as well as in age-related cancer [19, 20, 36], highlights the prescient nature of the observations made in the Tamm laboratory 15-20 years ago on the effects of IL-6 on the social behavior of cancer cells [reviewed in 21].

Thirty years with IL-6

The discovery of IL-6 in 1980 in my laboratory arose from within the interferon field [1]. It was only in 1986 with the confluence of B-cell stimulatory factor with interferon-β_2 [4] and then in 1987 with the confluence of hepatocyte stimulating factor with those cytokines that the full range of IL-6 biological properties came to be appreciated [5]. It is gratifying that many of the early investigations in the 1980s and 1990s dealt with issues that continue to attract considerable interest today. Indeed, it has been a privilege to be associated with the IL-6 field from its very beginning and to witness the broad march of research in this area of biomedical science during the last thirty years.

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It has been a pleasure to have begun to collaborate with Dr. Andrzej Mackiewicz of Poznan and Dr. Aleksander Koj of Krakow in June 1988 just as the IL-6 field was undergoing an explosive growth. The very first meeting dedicated to all aspects of research on IL-6 was organized by Dr. Mackiewicz in Połczyn-Zdrój in June 1988. By 1992-1993, when it became

clear that IL-6 was only one member of the larger group of IL-6-type cytokines, Drs. Mackiewicz, Koj and I collaborated on organizing the first ever conference for the New York Academy of Sciences in Eastern Europe in June 1994 in Poznan. The scientific and personal rewards flowing from these collaborations and those early years continue to enrich us today. I thank both Dr. Mackiewicz and Dr. Koj for their thoughtful conversation and enthusiastic support over the years. Research in the author's laboratory was supported by Research Grant HL-087176 from the National Institutes of Health.

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