Review

Immunoglobulin Expression in Non-Lymphoid Lineage and Neoplastic Cells

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It has traditionally been believed that the production of immunoglobulin (Ig) molecules is restricted to B lineage cells. However, immunoglobulin genes and proteins have been recently found in a variety of types of cancer cells, as well as some proliferating epithelial cells and neurons. The immunoglobulin molecules expressed by these cells consist predominantly of IgG, IgM, and IgA, and the light chains expressed are mainly kappa chains. Recombination activating genes 1 and 2, which are required for V(D)J recombination, are also expressed in these cells. Knowledge about the function of these non-lymphoid cell-derived immunoglobulins is limited. Preliminary data suggests that Ig secreted by epithelial cancer cells has some unidentified capacity to promote the growth and survival of tumor cells. As immunoglobulins are known to have a wide spectrum of important functions, the discovery of non-lymphoid cells and cancers that produce immunoglobulin calls for in-depth investigation of the functional and pathological significance of this previously unrecognized phenomenon. (Am J Pathol 2009, 174:1139–1148; DOI: 10.2353/ajpath.2009.080879)

V(D)J recombination and production of Ig molecules traditionally occur only in B lymphocytes and plasma cells. However, recently many researchers have reported Ig expression in non-lymphoid cells, including epithelial cancer cells and proliferating epithelial cells and central neurons. This intriguing new discovery makes possible the potential identification of novel immunoglobulin function in normal and abnormal physiological states of many cell types and may unveil new facets of immune and regulatory function. This review will focus on this new phenomenon, including its molecular mechanism, and the biological function of Ig expression in non-lymphoid cells.

Immunoglobulin Structure

Immunoglobulin biology was originally characterized in lymphoid cells. Immunoglobulin molecules are composed of two identical light (L) chains of molecular weight 22,500 and two heavy (H) chains of molecular weight 50,000 to 75,000, which are linked by noncovalent interactions and disulfide bridges to form a structure with twofold symmetry. Each chain is characterized by a unique (or nearly unique) sequence in their C-terminal region that contributes to determining antigen specificity. Immunoglobulin L chains are classified into two isotypes (or classes), κ and λ . The relative proportions of κ and λ vary considerably with species, from a κ to λ ratio of 65% to 35% in humans to a ratio of 97% to 3% in mice.¹ There are five immunoglobulin isotypes, IgG, IgM, IgA, IgD, and IgE. Although each isotype can possess either κ or λ light chains, their H chains (called γ , μ , α , δ , and ϵ respectively) are all different, and each is specific to its immunoglobulin class.² Two isotypes, IgA and IgM, may form multimers through disulfide bridges between H chains of individual immunoglobulin molecules.¹

Immunoglobulin Function

Immunoglobulins can be both membrane-bound and secreted. Secreted immunoglobulins constitute serum antibodies. The membrane-bound immunoglobulins, together with the two transmembrane proteins Ig- α and Ig- β , comprise the B cell antigen receptor, which plays a central role in determining the fate of B cells.³ Cross-linking of the B cell receptor by antigen activates multiple signal pathways inside the B cell, such as the phospholipase C- γ 2, phosphoinositide 3-kinase, and GTPases pathways. These intracellular molecular events contribute to B lymphocyte proliferation, deletion, anergy, receptor editing, and survival.⁴

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For secreted immunoglobulins, limited proteolysis results have shown that immunoglobulin molecules are composed of two copies of a variable Fab region that contains an antigen binding site, and a relatively constant Fc region that interacts with effector molecules such as complement proteins and Fc receptors (FcRs).¹ There is a FcR specific for each antibody class: Fc γ R binds IgG, Fc α R binds IgA, Fc ϵ R binds IgE, Fc μ R binds IgM, and Fc δ R binds IgD.⁵ FcRs are expressed on many immune effector cells, such as monocytes, neutrophils, eosinophils, basophils, mast cells, NK cells, B cells, T cells, and tissue macrophages.⁶ Through interaction with the Fc region of immunoglobulins, FcRs mediate the following effector responses: phagocytosis, endocytosis, antibody-dependent cell-mediated cytotoxicity, the release of inflammatory mediators, and the regulation of B cell activation and antibody production.^{6–8}

Phagocytosis is a process whereby microbial particles are engulfed, internalized into acidified cytoplasmic vesicles named phagosomes, and digested by lysosomal enzymes after fusion of the phagosomes with lysosomes.⁸ Phagocytosis and endocytosis are differentiated by the size of the particle that is ingested and degraded. In phagocytosis, particles of 1 μ m or greater in diameter are engulfed, while endocytosis describes the internalization of smaller antibody-antigen complexes.⁸ Endocytosis of immune complexes via FcR enhances antigen presentation by major histocompatibility class II-positive cells.⁵

FcRs present on cells like natural killer cells interact with antibody-bound target cells, causing the destruction of target cells by antibody-dependent cell-mediated cytotoxicity.^{6–8} Interaction of FcRs with antibody-antigen complexes has also been shown to trigger release of a variety of soluble mediators. These include inflammatory mediators like leukotrienes and prostaglandins, various hydrolytic enzymes, and multiple cytokines, including interferon- γ , interleukin-1, interleukin-6, and tumor necrosis factor- α .⁶ The FcRs on B cells were shown to deliver a dominant negative signal to B cells after cross linking, resulting in inhibition of both proliferation and Ig production.⁶

Antigen-antibody complexes can also activate the complement system, which mediates elimination of microorganisms and immune damage to the host.⁹ The complement component C1q binds with IgM and IgG immune complexes, thereby triggering the “classical complement pathway activation,” resulting in the generation of C3 and its proteolytic components.¹⁰

Immunoglobulin Diversity

To produce the vast number of antigen-specific immunoglobulin proteins required for adaptive immunity, lymphoid cells undergo genomic recombination. The immunoglobulin gene system is comprised of three separate gene loci known as L κ , L λ , and H chain, and each of these loci contains variable (V) and constant (C) genes.¹¹ The L κ , L λ , and H chain genes are located on human chromosomes 2,¹² 22,^{13,14} and 14^{15,16} respectively. For

light chains, the V region is encoded by two separate DNA segments, namely the V κ and J κ (or V λ and J λ) segments. Similarly, the V $_H$ region is encoded by three separate DNA segments, namely, the V $_H$, D, and J $_H$ segments.¹⁷

The variable region of immunoglobulin genes is assembled from component V, D, and J gene segments through V(D)J recombination.¹⁸ Recombination-activating gene (RAG) endonuclease is required *in vivo*^{19,20} and sufficient *in vitro*^{21,22} to initiate the cleavage phase of V(D)J recombination. RAG is composed of two enzymes, RAG1 and RAG2, and mice deficient either in RAG1 or RAG2 lack mature lymphocytes owing to their inability to initiate V(D)J rearrangement. RAG introduces DNA double-strand breaks specifically at the borders between two coding segments and their flanking recombination signal sequences. Recombination signal sequences consist of a highly conserved heptamer and nonamer, separated by a relatively nonconserved spacer of either 12 or 23 base pairs (bp).²³ For efficient recognition and double-strand break formation, RAG requires that one recombination signal sequence have a 12-bp spacer and the other a 23-bp spacer, a restriction referred to as the 12/23 rule.^{24,25} The resultant resolved double-strand breaks are repaired by ubiquitously expressed nonhomologous end joining proteins to generate coding and recombination signal sequence joints, yielding a unique antibody specificity.²⁶

There are two additional types of genetic alteration in Ig genes that contribute to Ig diversity, namely somatic hypermutation of V genes, and class switch recombination of Ig genes. Both of these processes take place in the germinal centers of lymphoid organs.^{27,28} Class switch recombination results in switching of the immunoglobulin isotype from IgM to IgG, IgE, or IgA by replacing the immunoglobulin C $_H$ gene to be expressed from C μ to C γ , C ϵ , or C α , respectively, without changing the antigen specificity. Each isotype determines the manner in which captured antigens are eliminated or the location where the immunoglobulin is delivered and accumulated.²⁹

Somatic hypermutation occurs when a germline immunoglobulin sequence is altered by the introduction of nucleotide changes during the lifetime of a B cell. Somatic hypermutation takes place in the V region of both H and L chain genes and accumulates massive point mutations in the V exon. B cells expressing high affinity Ig on their surface are then selected by limited amounts of antigens in a process called affinity maturation.³⁰ A putative RNA editing enzyme, activation-induced cytidine deaminase (AID), is required for both class switch recombination and somatic hypermutation in mouse and human.^{31–37} AID-deficient mice cannot produce IgG, IgA, or IgE antibodies; however, IgM is more abundantly produced under immunized or non-immunized conditions. Induced overexpression of AID in CH12F3-2 B lymphoma cells enhances class switch recombination, irrespective of cytokine stimulation.³³

Having reviewed the general structure, function, and diversity of Igs in lymphoid cells, the following text will focus on Ig expression in cancer cells and other non-lymphoid lineage cells.

Expression of Immunoglobulin Heavy Chains in Cancer Cells

The discovery of immunoglobulin heavy chain proteins and their corresponding genes in cancer cells is relatively recent. Since the first findings on this topic, a number of research groups have described findings that have opened the way for more extensive investigation of this novel area. The following paragraphs review the studies that contributed to this body of knowledge.

The immunoglobulin heavy chains expressed in cancer cells were found to consist mainly of γ , μ , and α chains. In 1998, Kimoto, using nested reverse transcription (RT)-PCR, detected gene transcripts for the heavy chain constant regions of IgM, IgD, IgG3, IgG1, IgE, and IgA and the T-cell receptor- α in five carcinoma cell lines, including SW1116 (colon adenocarcinoma), HEp2 (laryngeal squamous cell carcinoma), MCF-7 (estrogen receptor-positive mammary adenocarcinoma), MDA-MB-231 (estrogen receptor-negative mammary adenocarcinoma), and HC48 (pancreatic adenocarcinoma).³⁸ A human Ig heavy chain constant region was detected in hepatocellular carcinoma total RNA by use of cDNA microarray.³⁹ Ig heavy chain protein could also be detected by using two-dimensional electrophoresis in human nasopharyngeal carcinoma cell lines.⁴⁰

In 2003, Qiu et al reported that human cancers of epithelial origin including carcinomas of breast, colon, liver, and lung, could produce IgG.⁴¹ In this study they identified mRNA of IgG heavy chain in these tumor cells, and also found corresponding expression of IgG. Immunohistochemistry analysis showed this IgG was localized in the cytoplasm or on the plasma membrane of these cells. The culture supernatant of two cervical cancer cell lines (HeLa S3 and HeLa MR) was also positive for IgG, suggesting these cells were secreting this IgG.⁴¹ By cloning and sequencing the complementary-determining region 3 of IgG heavy chain, Qiu et al found monoclonality of the V-D-J recombination in HeLa S3 and HT-29 (a colon carcinoma cell line) and found that HT-29 had a V-D-J recombination sequence identical to HeLa MR.⁴¹

In a different study, Zheng et al found a novel immunoglobulin gene SNC73 by subtractive hybridization using cDNA of normal mucosal tissues and mRNA of colorectal cancer.⁴² This gene was mapped to human chromosome 14q32. Open reading frame prediction showed that SNC73 encoded a peptide identical to the constant region of an IgA molecule in the carboxyl-terminus. Their group later confirmed expression of SNC73 in normal epithelial cells and corresponding cancer cells, including colorectal cancer, gastric cancer, breast cancer, lung cancer, and liver cancer.^{43–45} However there were no significant differences in expression of SNC73 among gastric cancer, breast cancer, lung cancer, and liver cancer when compared with non-cancerous tissues.⁴⁵

In 2006, Babbage et al analyzed immunoglobulin heavy chain (IgH) gene expression by nested RT-PCR in six well-defined breast cancer cell lines (BT474, MDA-

MB-231, MCF-7, SKBR3, T47D, and ZR75-1).⁴⁶ IgH gene transcripts were identifiable in four of these six cell lines. To further explore this tumor-associated IgH gene expression, they analyzed IgH gene transcripts in sorted EpCAM⁺ (epithelial cell adhesion molecule positive) single cells and found that the frequency of single cells expressing IgH gene was significant, averaging about 32%. This revealed that the IgH gene was expressed only in a fraction of each cell line and not in all tumor cells, indicating that these rearrangements likely occurred in a minority of cells after transformation. It was clear that the IgH genes in these breast cancer cell lines were expressed as pre- and post-switched transcripts, as both IgG and IgA were expressed in SKBR3 and both IgM and IgG were expressed in ZR75-1. However only IgM was detected in MDA-MB-231, and IgG was detected in T47D. Moreover, by cloning the amplified IgH gene product, they found that in five of six of the identified IgH genes, somatic mutations were apparent with no intraclonal variation, indicating cessation of mutational activity. It was also of interest that a pseudogene V_{3-41} was found to be expressed in T47D, as its expression in normal B cells would lead to a dysfunctional cell that would be aborted.⁴⁶

In 2007 Chen et al demonstrated IgG heavy chain and κ light chain expression in several kinds of carcinoma cells, as well as in some non-transformed proliferating cells⁴⁷ (Figure 1). In the same year, another group reported IgA heavy chain expression in human epithelial cancer cell lines.⁴⁸ By alignment of the database of expressed sequence tags (dbEST) to the IgA constant region from B lymphocytes, Zheng et al found that most expressed sequence tags originate from B lymphocytes, but many expressed sequence tags were derived from human epithelial cancers, including cancers of the lung, breast, colon, stomach, kidney, and so on. Subsequently, they detected the expression of IgA in several epithelial cancer cell lines, including cervical cancer (HeLa), nasopharyngeal carcinoma (CNE1), gastric cancer (MGC), breast cancer (MCF-7), and colon cancer (SW480). They also detected IgA in culture supernatants of these cancer cell lines, and identified expression of the J chain and secretory component proteins. On the basis of these findings, they hypothesized that cancer derived IgA may combine with J chain protein to form secretory IgA (SIgA).

In 2008, Zhu et al reported that oral epithelial tumors, including benign pleomorphic adenomas, malignant tumors including squamous cell carcinoma and mucoepidermoid carcinoma (and other malignant tumors) showed cytoplasmic, plasma membrane, and secreted IgG and IgA proteins by immunohistochemical analysis. These proteins were also found in some normal gland epithelial cells or squamous cells adjacent to these tumors. At the same time, rearranged Ig γ 1 gene transcripts were also identified in pleomorphic adenoma cells and some normal gland epithelia adjacent to tumor. Moreover, in each separate case, the V-D-J region sequences revealed identical V-D-J rearrangement.⁴⁹

Figure 1. IHC shows existence of IgG heavy and light chains in cancer cells. **A:** Expression of the immunoglobulin heavy chain in carcinomas using a rabbit anti-human IgG γ chain specific antibody. **B:** Expression of the immunoglobulin light chain in carcinomas using a mouse anti-human κ chain antibody. Brown is the positive signal. **1)** Lung squamous cell carcinoma. **2)** Hepatocellular carcinoma. **3)** Cervical squamous cell carcinoma. **4)** Prostate carcinoma. **5)** Invasive ductal breast carcinoma. **6)** Relatively normal breast tissue in a breast benign disease. Reproduced from Chen and Gu (FASEB J 2007, 21:2931–2938)⁴⁷ with permission of the Federation of American Societies for Experimental Biology. Scale bars = 20 μ m.

Expression of Immunoglobulin Light Chains in Cancer Cells

The light chains expressed in cancer cells have been reported to be predominantly κ chains. Cao et al detected Ig κ chain expression in nasopharyngeal carcinoma cell lines by RT-PCR, Western blot, and fluorescence-activated cell sorting.⁵⁰ They also detected mRNA for the kappa chain constant region in abnormal human uterus cervical epithelial cells, including cervicitis, cervical intraepithelial neoplasia, and invasive cervical carcinomas. Moreover, the expression of Ig κ was found to be up-regulated in cervical epithelia with dysplasia and carcinoma, as compared with cervicitis.⁵¹

Ig κ was also found to be expressed in other tumor cells such as breast cancer, lung cancer, liver cancer, prostate cancer,⁴⁷ colorectal carcinoma⁵² and gastric cancer.⁵³ Although Ig λ was reported in one study to be co-expressed with Ig κ in gastric cancer by immunohistochemistry,⁵³ another research group found no evidence of λ chain expression in other epithelial cancer cell lines.⁵²

The cancer cell lines and tissues that express Ig heavy chain and/or light chain are summarized in table 1.

Molecular Mechanisms of Immunoglobulin Expression in Cancer Cells

RAG1 and RAG2 are indispensable for V(D)J recombination. It is intriguing that both RAG1 and RAG2 mRNA and proteins have been detected in Ig positive cancer cell lines, including lung cancer, colon cancer, uterus cervical cancer,⁴¹ liver cancer, prostate cancer,⁴⁷ gastric cancer, breast cancer, and nasopharyngeal carcinoma.⁴⁸ AID, which is essential for both class switch recombination and somatic hypermutation, was also reported to be expressed in six breast cancer cell lines by nested RT-PCR,⁴⁶ although attempts to detect AID in other epithelial cancer cell lines by means of RT-PCR and Western blot⁴⁸ were not successful.

Histone acetylation and germ-line transcription correlate strongly with an open or an accessible chromatin structure thought to be permissive for V(D)J recombination.⁵⁴ Both sense and antisense germ-line transcription have been shown to correlate well with IgH V(D)J joining^{55,56} and treatments that activated germ-line gene transcription increase the frequency of Ig gene rearrangement.^{57,58} It is also known that isotype switch in B cells is preceded by induction of sterile germ-line transcripts from I μ exons, which lie upstream of the coding region for the immunoglobulin heavy chain isotype.⁵⁹ It is of interest that both I γ -C γ and I α -C α germ-line transcripts were detected in these Ig positive epithelial cancer cell lines (breast cancer, liver cancer, cervical cancer, prostate cancer, nasopharyngeal carcinoma, gastric cancer, and colon cancer).^{46–48}

The generation of B lineage cells together with Ig production is controlled by a regulatory network that includes the cytokine receptors Flt3 and interleukin-7R, and the transcription factors PU.1, Ikaros, E2A, EBF, and Pax5.^{60–66} B cell development in either E2A^{−/−} or EBF^{−/−} mice is arrested at its earliest stage in the absence of RAG expression and D μ to J μ rearrangements at the IgH locus,^{67–69} and ectopic expression of E2A and EBF, together with RAG1 and RAG2 activates D μ to J μ rearrangement in non-lymphoid cells.^{70,71} Several regulatory elements have been described in the RAG locus. In addition to the RAG1 and RAG2 promoters,^{72,73} there are three distinct enhancers: the proximal enhancer (Ep), the distal enhancer (Ed), and Erag.^{74–76} E2A transcription factors bind to Erag *in vivo* and can transactivate Erag-dependent reporter constructs in cotransfected cell lines.⁷⁵ E47 can activate RAG1 expression even in fibroblasts.⁷⁷ Ikaros activates expression of the RAG locus by binding to RAG1 promoter, Ep, Ed, and Erag.⁷⁸ Pax5 has been shown to bind and activate RAG2 promoter.^{73,79} E2A can also bind and activate the IgH enhancer and kappa light chain intronic enhancer, which are important to germ-line transcription and rearrangements.^{77,80}

Table 1. A Summary of Cancer Cell Lines and Tissues that Express Immunoglobulin Heavy Chains and/or Light Chains

Cancer cell lines or tissues	Ig heavy chain expression	Ig light chain expression	References
MCF-7 (breast cancer)	γ , μ , α , δ and ϵ chains	N.D.	38, 41, 48
MDA-MB-231 (breast cancer)	γ , μ , α , δ and ϵ chains	N.D.	38, 46
SKBR3 (breast cancer)	γ and α chains	N.D.	46
T47D (breast cancer)	γ chain	N.D.	46
ZR75-1 (breast cancer)	γ and μ chains	N.D.	46
Bcap37 (breast cancer)	α chain	κ chain	42, 52
SW1116 (colon cancer)	γ , μ , α , δ and ϵ chains	N.D.	38, 42
LOVO (colon cancer)	γ and α chains	κ chain	41, 52
HT-29 (colon cancer)	γ chain	N.D.	41, 83
SW480 (colon cancer)	α chain	κ chain	42, 44, 48, 52
SW620 (colon cancer)	α chain	N.D.	42
WIDR (colon cancer)	α chain	N.D.	42
HeLa (cervical cancer)	γ and α chains	κ chain	42, 48, 52
HeLa S3 (cervical cancer)	γ chain	κ chain	41, 47
HeLa MR (cervical cancer)	γ chain	N.D.	41
MGC (gastric cancer)	α chain	N.D.	48
MKN28 (gastric cancer)	α chain	N.D.	42
SGC791 (gastric cancer)	α chain	N.D.	42
BCL-7402 (liver cancer)	γ chain	κ chain	41, 47
SMMC7221 (liver cancer)	α chain	κ chain	42, 52
A549 (lung cancer)	γ chain	κ chain	41, 47
PAA (lung cancer)	α chain	N.D.	42
HEp2 (laryngeal cancer)	γ , μ , α , δ and ϵ chains	N.D.	38
HC48 (pancreatic cancer)	γ , μ , α , δ and ϵ chains	N.D.	38
CaOV3 (ovarian cancer)	γ chain	N.D.	41
PC3 (prostate cancer)	γ chain	κ chain	47
CNE1 (Nasopharyngeal cancer)	α chain	N.D.	48
HNE2 (Nasopharyngeal cancer)	N.D.	κ chain	50
Tca8113 (tongue cancer)	α chain	N.D.	42
Breast cancer tissues	γ and α chains	κ chain	41, 43, 45, 47
Colon cancer tissues	γ and α chains	N.D.	41, 42, 43, 45
Gastric cancer tissues	γ and α chains	κ and λ chains	43, 45, 53
Liver cancer tissues	γ and α chains	κ chain	41, 43, 45, 47
Lung cancer tissues	γ and α chains	κ chain	41, 43, 45, 47
Cervical cancer tissues	γ chain	κ chain	47, 51
Prostate cancer tissues	γ chain	κ chain	47
Oral cancer tissues	γ and α chains	N.D.	49

N.D., not determined.

It has been shown that Pax5 could induce large scale contraction of the IgH locus, promoting long range V_H to DJ_H recombination by juxtaposition of distal V_H genes next to the proximal D_HJ_H rearranged gene segment.⁸¹ Recently Ikaros was also reported to control accessibility and compaction of the IgH locus.⁷⁸ These transcription factors not only activate Rag expression, but also control the accessibility and compaction of Ig variable genes.

Recently Geng et al detected expression of Pax5 in a human colon cancer cell line (SW480), and EBF expression in five epithelial cancer cell lines by means of RT-PCR.⁵² These cell lines included colon cancer (SW480 and LOVO), uterine cervical cancer (Hela), breast cancer (Bcap-37), and liver cancer (SMMC-7721). However, whether the expression of these transcription factors exists in other Ig-positive cancer cells needs further exploration. Moreover, it is clear that the functional consequences of expression of these transcription factors will require further study to determine the molecular mechanism of Ig expression in cancer cells.

In one model, a pathway by which Ig expression is mediated in tumor cells has been worked out. Cao et al reported that latent membrane protein 1 encoded by Epstein-Barr virus up-regulated Ig κ expression in a

nasopharyngeal carcinoma cell line via NF- κ B and AP-1 pathways.^{50,82} By using inhibitors of JNK and NF- κ B, up-regulation of the expression of kappa was inhibited. Latent membrane protein 1-positive nasopharyngeal carcinoma cells expressing the dominant-negative mutant of I κ B α (DNMI κ B α) or of c-Jun (TAM67) exhibited significantly decreased kappa production compared with their parent cells. However, whether similar pathways exist in other epithelial cancer cells remains to be investigated. The hypothetical mechanism of V(D)J recombination is illustrated in Figure 2. However other possible mechanisms may exist.

Biological Function of Immunoglobulin Expression in Cancer Cells

The literature regarding the biological function of Ig expression in cancer cells is very limited. In 2003, Qiu et al reported that by using either antisense oligodeoxynucleotide (ASODN) or anti-human IgG antibody, blockade of tumor-derived IgG increased programmed cell death and inhibited growth of cancer cells *in vitro*.⁴¹ In addition, administration of anti-human IgG antibody also sup-

Figure 2. The hypothetical molecular mechanism of V(D)J recombination in non-lymphoid cells. **A:** A possible mechanism of V_H to D_J_H recombination in non-lymphoid cells. **B:** Schematic depiction of rearrangement in the human $Ig\kappa$ locus. NF- κ B binds to its cognate site in $iE\kappa$ and induces germ line transcription (GLT) of the $J\kappa$ segments, making these segments accessible for recombination with upstream $V\kappa$ segments. RSS, recombination sequence signal. Kde, κ -deleting element.

pressed the growth of an IgG-secreting carcinoma cell line (HeLa MR) in immunodeficient nude mice. Therefore, they concluded that IgG secreted by cancer cells had some unidentified capacity to promote the growth and survival of tumor cells.

In 2006, Deng et al found that after transfection of the antisense vector of immunoglobulin heavy chain complementary-determining region 3 (specific to HT-29), the expression level of Ig gene segment in HT-29 cells was significantly decreased, and both cell growth inhibition and apoptosis were induced.⁸³ In 2007, Zheng et al showed that by using mouse anti-human Ig α antibody, blockade of cancer-derived Ig α suppressed the growth, viability and capacity for proliferation of cancer cells (HeLa and CNE1).⁸⁴ Furthermore, they demonstrated that blockade of cancer-derived Ig α decreased the access percentage of S phase from the early mitosis of synchronized cancer cells. These results support the concept that cancer-derived Ig is a growth promoter of cancer cells.

There is also some evidence of possible clinical significance of Ig expression in epithelial cells. Li et al showed that the expression of kappa constant region mRNA was markedly increased in uterine cervical epithelia with dysplasia and carcinoma, as compared with cervicitis.⁵¹ They concluded that the aberrant expression of the immunoglobulin kappa light chain constant region in dysplastic and cancerous cervical epithelial cells may serve as a marker for malignant cell transformation.

Due to limited literature about the function of Ig expressed in cancer cells, it is still a question whether cancer derived Ig has similar function as normal Ig, like complement activation, binding to FcRs, antibody-dependent cell-mediated cytotoxicity, and inducing phagocytosis. The antigen specificities of cancer derived Ig are also unknown. Those questions provide directions for future research.

It has been known for many years that the growth of tumor grafts can be enhanced by immune reactions, including antibody production.⁸⁵ Prehn suggested that weak immune reactions against primary tumors stimulate, rather than suppress, cancer growth.^{86,87} In some models, Igs that are either produced actively or administered passively have been shown to enhance the growth of tumor transplants.^{88,89} Although the exact mechanism of antibody enhancement of tumor growth remains unclear, it has been hypothesized that antibodies may play such a role by blocking target epitopes on the cancer cells.⁹⁰ Hellstrom et al suggested that the ability of lymphocytes to eliminate their targets may be diminished *in vivo* by serum "factors" that protect the neoplastic cells specifically (akin to enhancing antibodies) or nonspecifically.⁹¹ Sjogren suggested that the blocking factor in sera from tumor-bearing animals is an antigen-antibody complex, capable of binding to target cells and/or reacting with lymphocytes immune to their antigens, thus blocking the lymphocyte reactivity.⁹² In light of recent discoveries, it is not illogical to assume that one source of tumor-protecting Ig could be the cancer cells.

Immunoglobulin Expression in Epithelial Cells and Neurons

Besides cancer cells, Ig has also been found in human epithelial cells and normal mouse neurons. IgG was reported in some epithelial cells of human normal lung tissues,⁴¹ myoepithelium of breast fibroadenoma tissues, and hepatocytes of liver cirrhosis tissues.⁴⁷ The κ light chain was found to be expressed at a low level in epithelial cells from chronic uterine cervicitis tissues.⁵¹ A novel immunoglobulin gene SNC73 and κ light chain were found to be expressed in epithelial cells from a variety of normal tissues, including colorectal, gastric, breast, lung, and liver tissues.^{43,45} However the functions of Ig molecules in epithelial cells are still unknown.

Recently IgG heavy chain and κ light chain were reported to be expressed in normal mouse neurons.⁹³ The presence of immunoglobulin molecules in cerebrospinal fluid and in brain neurons has been reported for more than ten years⁹⁴⁻⁹⁸ and it was originally suspected that these neurons were taking up immunoglobulin proteins from the extracellular fluid. However, in 2007, Huang et al reported the presence of rearranged immunoglobulin γ chain and κ chain transcripts in adult mouse brain neurons.⁹³ They also identified IgG proteins in the neuronal cytoplasm, including axons and dendrites. Finally, sulfur-35 and iodine-125 pulse-labeled immunoprecipitation assays gave additional confirmation of IgG production in neurons in the brain.

The brain is an immune privileged organ and the significance of IgG expression in neurons under physiological conditions is not established yet. Intravenous immunoglobulin has been increasingly used for the treatment of various autoimmune and systemic inflammatory diseases, such as Kawasaki disease, dermatomyositis, multiple sclerosis, and graft versus host disease.⁹⁹ The beneficial effects of intravenous immunoglobulin can be explained by neutralization, accelerated clearance

and prevention of Fc γ -receptor binding of autoantibodies.^{100,101} Recently intravenous immunoglobulin was reported to protect the brain against the experimental ischemia and reperfusion by preventing complement-mediated neuronal cell death, even in relatively small doses.¹⁰² Intravenous immunoglobulin may protect neurons in stroke by inhibiting multiple components of inflammation, including complement fragments, pro-inflammatory cytokine production, and leukocyte cell adhesion.¹⁰³ Whether Ig molecules produced by normal neurons have a protective effect for neurons needs further investigation. We hypothesize that Ig produced by central neurons may play a pivotal role in protecting the neurons from various insults.

Discussion and Conclusion

Although Ig genes, proteins, and RAG1/RAG2 are reported to be expressed in cancer cells, some caveats about the published results should be noted. Firstly, it is generally accepted that certain established tumor lines (eg, variants of the HeLa line) are the result of a cell line mix-up. None of the manuscripts published on this topic have addressed this point. To exclude the contamination of B lymphocytes in the cancer cell lines, some researchers performed flow cytometry using CD19 and/or CD20 antibodies.^{41,46–48} They reported that there were no CD19- or CD20-positive cells in cancer cells and the source of Ig must be these cancer cells. However detection of Ig expression in a single cell was not performed except in six breast cancer cell lines.⁴⁶ Secondly, the finding that antisense Ig mRNA interfered with tumor growth *in vitro* may be explained by a siRNA-mediated but Ig-nonspecific effect, eg, via the induction of proliferation-inhibiting cytokines. Further research should be performed to understand the biological function of Ig molecules in cancer cells.

There are also certain conflicts among the published data. Babbage et al did not detect Ig proteins in cancer cell lines with flow cytometry,⁴⁶ whereas other researchers reported detection of Ig proteins with a variety of methods, including Western blot, immunofluorescence, flow cytometry, and enzyme-linked immunosorbent assay.^{41,47,48,50} One explanation could be that Ig proteins produced by cancer cells are aberrantly glucosylated and cannot be recognized by available antibodies. The other explanation could be that Ig proteins are expressed at a very low level in cancer cells and the reported detection of Ig proteins was caused by antibody cross reaction with bovine Ig. The latter explanation seems less likely as Ig proteins can be detected in cancer cells in the absence of fetal bovine serum in the culture medium.^{44,48}

Another controversy is that Babbage et al detected AID expression in six breast cancer cell lines,⁴⁶ whereas other research groups failed to detect AID expression in cancer cell lines including breast cancer.⁴⁸ Whether AID is expressed at a very low level in cancer cells needs further investigation.

More studies are needed to answer a number of key questions regarding this new discovery. The molecular

mechanism, biological function and clinical significance of Ig production in non-lymphoid cells, particularly cancer cells, warrants in-depth investigation. It will be intriguing to discover whether Igs derived from non-lymphoid cells have the same traditional function as normal Igs produced by lymphoid lineage cells, whether these Igs can bind to their parent cells, and what are the antigen specificities of these cancer derived Igs. Attention should be paid to transcriptional control of Ig expression and analysis of the character of rearranged DNA sequences in cancer cells for comparison with sequences from normal B lymphocytes. By doing so we hope to understand whether the same mechanism that governs Ig production in both B lymphocytes and cancer cells, or whether the mechanism governing the latter is distinctly different.

Since there appears to be association between Ig expression and cell transformation, the question as to whether Ig expression is a cause or a result of cell transformation awaits investigation. It also remains to be seen whether there is any correlation between Ig expression level and tumor differentiation. It would be of interest to examine whether Ig expression can be used as a diagnostic and prognostic marker or a therapeutic target. It is likely that the answers to these questions will give us new clues about carcinogenesis and cancer therapy. Moreover, Ig expression in central neurons suggests an association with Igs and normal neuron development. In addition, as the brain is an immune-privileged organ, the possibility that there is participation of central neuron produced Igs and Igs produced by non-lymphoid cells in immune protection of neurons and of other cell types is yet another fundamental question in the biology of immunoglobulins. As Ig not only plays a pivotal role in the physiology and pathology of the human body, but also is a crucial tool in diagnosis and treatment of diseases by pathologists and other physicians, the possible unveiling of an unknown aspect of this class of molecules warrants urgent attention.

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