Review

Immunoglobulin Expression and Its Biological Significance in Cancer Cells

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It is generally believed that the expression of a gene is restricted "within the right place and at the right time". This principle has long been considered applicable as well to the expression of immunoglobulin (Ig) lymphocytes of B cell lineage. However, increasing evidence has shown Ig "paradoxically" expressed in malignant tumors of epithelial origin. We reviewed the recent progress in the study of cancer-derived Ig, and also discussed its mechanisms and possible functions, trying to arouse interest and attention to those working in the field of immunology and oncology. *Cellular & Molecular Immunology*. 2008;5(5):319-324.

Key Words: immunoglobulin, carcinoma, recombination, class switch, signal transduction

Introduction

Though classic immunology implies that differentiated B cells are the unique source of Ig, Cao et al. cloned the JC region of human Igκ light chain from a genomic DNA library of human nasopharyngeal carcinoma (NPC) cell line CNE2 in 1991 (1). Since then, increasing evidence has shown Ig "paradoxically" expressed in malignant tumors of epithelial origin. This phenomenon, though hitherto not yet universally acknowledged, has indeed become a great challenge to the classical dogma of contemporary immunology.

Immunoglobulin expression in B lymphocytes

Immunoglobulin (Ig) molecule is composed of two identical light chains and two identical heavy chains. The poly-peptides are linked by disulfide bridges connecting each light chain to heavy chain and linking the two heavy chains together (2, 3).

During B lymphocyte development and maturation, the process of Ig expression and secretion is of multiple stages under successive microenvironments (4-6). Important genomic alteration events in this process including V(D)J recombination and class switching are regulated precisely and tightly, in order to ensure the development of a normal immune system, and to prevent potentially oncogenic processes, such as translocations, caused by errors in the recombination or switching processes (7-9).

V(D)J recombination is initiated by the recombination activating genes (Rag)1 and Rag2 proteins in developing B lymphocytes. The exons which encode Ig variable regions are assembled from germline variable (V), diversity (D), and joining (J) gene segments: V_H, D_H, and J_H gene segments for Ig heavy chain; V_L and J_L gene segments for Ig light chain (4, 5).

B lymphocytes further diversify their repertoire in the germinal centers of lymphoid organs through class switch recombination (CSR). CSR is a region-specific rearrangement process triggered by the activation-induced cytidine deaminase factor (AID), which is required for both somatic hyper-mutation of V genes and CSR. CSR is achieved by looping-out deletion of the genomic DNA between the recombined S regions and the switch circle (SC). By CSR, B lymphocytes can change the constant region portion of the heavy chain, shifting the class of expressed antibody from IgM to IgG, IgA, IgD or IgE to confer distinct antibody effector functions (5, 10).

V(D)J recombination and CSR are generally believed to occur only in lymphocytes, where various receptor loci must become accessible at the appropriate stage of differentiation and be regulated in the context of lineage specificity and developmental stage specificity (IgH genes before IgL genes) (6, 11), which yields the classic view that B cells are the only source of Ig.

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Received Jul 10, 2008. Accepted Aug 31, 2008.

Ig expression in cancer cells

In 1991, from a genomic DNA library of CNE2, Cao et al cloned a transforming gene named Tx (GenBank accession number: AF279037), which turned out to be the JC region of human Igκ light chain (1). Later, Ig molecules composed of α heavy chain and κ light chain were detected in cell lines MCF-7 (breast cancer), MGC (gastric cancer), HeLa (cervical cancer), SW480 (colon cancer) and HNE-2 (nasopharyngeal cancer) (12). The finding that Ig could be expressed in carcinoma cells challenged the classic view that in non-lymphocytes, the genes encoding Ig are not to be transcribed.

In 1998, by RT-PCR, Kimoto demonstrated expression of Ig gene transcripts of IgM, IgD, IgG3, IgG1, IgE, and IgA in several other cancer cell lines: 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 (13), further confirmed Ig expression in cancer cells.

With high-throughput genomics and proteomics research methodology, even more incidences of Ig expression in cancers are being reported (14, 15), and some researchers testified Ig expression in corresponding cancer cell lines and tissues (16-20). It was found that these cancer cells or tissues were of a wide range of tissue origins. They came from epithelial tissues of respiratory tract, digestive tract and reproductive tract. The most recent studies by Qiu suggested that Ig could be found in oral epithelial tumor cells (21), and even in adult mouse brain neurons or isolated neonatal mouse neurons (22).

The Ig expressed in cancer cells or tissues included basically all kinds of isotypes, among heavy chains, α chain for IgA and γ chain for IgG were the mostly identified; but in light chain, only κ chain but not λ chain was confirmed. However, research groups may draw different conclusions among cancer cells of different tissue origins, implying complicated and varied Ig expression procession in cancer cells.

The database of expressed sequence tags (dbEST), a division of GenBank, contains sequence data and other information on single-pass cDNA sequences, or expressed sequence tags (ESTs). The dbEST database can be used to identify in which cell types a given gene is expressed. Alignment of human dbEST to IgCα region (constant region of α heavy chain) from B lymphocytes showed that most ESTs originate from B lymphocytes, but many ESTs are derived from human epithelial cancers, including cancers of the lung, breast, colon, stomach, kidney, etc. These ESTs have very high score value and identity (23).

Interestingly, cancer cells also secrete assembled Ig protein out of cell membrane. By cultivating MCF-7, MGC, HeLa, SW480 and HNE-2 cell lines (as described before) in serum-free culture medium, ELISA demonstrated existence of Igα heavy chain in the culture supernatant. From HeLa cell culture supernatant, purified Ig was found to contain Igα heavy chain and Igκ light chain identified by Western blot (12). These results provide the hints that in addition to Ig protein production, cancer cells are also capable of Ig secretion. Other supportive evidence has been reported by Qiu et al. By ELISA, they found IgG protein in the culture supernatant of two cervical cancer cell lines HeLa MR and HeLa S3, which suggested IgG secretion (18).

Recently, researchers also found Ig components in conditioned medium of lung cancer primary cell cultures by proteomics analysis (24). In the serum and tissue of lung squamous carcinoma, different levels of Ig protein fragments were detected (25, 26), which might present evidences for Ig secretion.

V(D)J recombination, class switching and CDR3 sequence in cancer cells

In normal B lymphocytes, before Ig expression, V(D) J gene fragments have to be recombined at gDNA level. The fact that cancer cell could generate Ig protein gives us a clue that the process of V(D)J recombination may also be initiated in cancer cells.

Peter et al. showed that rearrangement of IgH and IgL genes in non-lymphocytes was inducible by ectopic expression of E2A (one of Igκ and IgH enhancer) or early B cell factor (EBF) with RAG (27), provided the possibility that appropriate gene locus for recombination may be accessible in cancer cells, too.

In one of our studies, by PCR and sequencing of the Igα gDNA VDJ conventional gene segments, we identified that nasopharyngeal cell line CNE1 had recombined Igα gene structures. We also found RAG1 and RAG2 expression in MCF-7, MGC, HeLa, SW480 and HNE-2 cell lines as described above. Our results proved that cancer cell were capable of processing recombination. However, in CNE1 cell, IgSα (switch region of alpha heavy chain) gene keeps complete germline DNA construction, and AID (necessary for class switch) has not been found in both protein and mRNA levels (23). It could be inferred that Ig genes in these cancer cells have undergone V(D)J recombination but no class switching. Thus, the process of Ig expression in normal cancer cells is not completely the same as that in normal B lymphocytes.

The complementarity determining region (CDR) is a short amino acid sequence in the variable domains of antigen receptor (surface Ig on B cells or T cell receptors) proteins that complements an antigen, provides the receptor with its specificity for a particular antigen. Each polypeptide chain of an antigen receptor contains three CDRs (CDR1, CDR2 and CDR3). These regions are sometimes referred to as hypervariable domains because they are associated with most sequence variation. Among them, CDR3 shows the greatest variability as it differs with each recombination (28, 29). Therefore its sequences in different cancer cell lines would be expected to be diverse. We used three conventional primers to analyze the CDR3 hypervariable region of the cell lines including Raji (B-lymphoma), SW480 and CNE1. The

results showed that different cancer cell lines produced diverse Ig CDR3 clonalities, which did not exist in present NCBI database (23).

Other research groups also have similar but not identical results. Qiu et al. by demonstrating RAG-1 and RAG-2 expression in a series of IgG expressing cell lines: MCF-7 (breast cancer), HT-29 and LOVO (both colon cancer), HeLa MR and HeLa S3 (both cervical cancer), A549 (lung cancer), CaOV3 (ovarian cancer), proved the recombination ability of a variety of cancer cells. CDR3 sequencing results demonstrated monoclonality of the VDJ recombination in HeLa S3 and HT-29, although occasionally it contained single nucleotide deletion, insertion or mutation, whereas the Ig in HeLa MR and A549 did not. Interestingly, HT-29 line had identical CDR3 sequence as HeLa MR. Though the precise antigenic specificity of the cancer-derived Ig was unknown, lung and breast cancer-derived IgG was capable of binding smooth and striated muscles. It suggested that IgG from the two different cancer cell lines may be auto-antibodies reacting to an identical antigen. Moreover, the Ig V-D-J sequence of HT-29 was partially homologous with that of an autoantibody (GenBank No. Y17928) (18).

Zheng et al. identified RAG1 and RAG2 protein in LOVO and SW480 (both colorectal cancer), HeLa (cervical cancer), Bcap-37 (breast cancer), SMMC-7721 (human hepatoma) cell lines, in which the expressions of IgA1 heavy chain and Igκ light chain were detected. The mRNA of EBF was also detected in these cell lines, and immunoglobulin transcription factor Pax5 was only expressed in SW480 cells, but no expression of E2A was observed in all the five cell lines (20). However, neither Qiu's nor Zheng's publications mentioned whether AID or other CSR evidence was found in these cell lines.

Babbage et al. gave some clues about CSR in cancer cells in six well-characterized breast cancer cell lines (BT474, MDA-MB-231, MCF-7, SKBR3, T47D, and ZR75-1). According to the results, V_H gene transcripts were identifiable by nested RT-PCR in four of six lines, most being potentially functional. V(D)J transcripts were observed in sequential cultures, indicating stable expression. In five of six identified V_H genes, somatic mutations were apparent with no intraclonal variation, indicating cessation of mutational activity. V_H transcripts were pre- and post-isotype switched, with activation of switch events evident from expressed germ-line switch transcripts in two of six lines. Most importantly, all of six cell lines expressed AID were essential for mutational and switch activity, suggesting CSR and modification of V_H genes in cancer cells (30).

Another finding about V(D)J recombination and CSR has been reported by Gu et al. In cancer cells A549 (lung cancer), BCL-7402 (liver cancer), HeLa S3 (cervical cancer) and PC3 (prostate cancer), mRNA of the IgHG1 (Ig heavy chain G1) constant region and Iγ-Cγ sterile transcripts were detected by nested RT-PCR, and Igγ and Igκ proteins were detected by immunofluorescence and Western blot, which confirmed IgG expression in these cell lines. The Sγ1/2-Sμ switch circle, and the expression of RAG1 and RAG2 were found, underlying the V(D)J recombination of IgH and IgL loci and CSR. AID expression could be detected too, but only in cell line HeLa S3 and positive control Raji (19).

All the conclusions drawn by different researchers imply that cancer cells actually initiate and undergo V(D)J recombination and CSR which are thought to occur in lymphocytes only. Their specific process may vary among different cancer cell types, thus showing complicated mechanism underlining Ig expression in cancer cells.

The possible function and immuno-activity of cancer cells derived Ig

It could be speculated that constitutive AID activation in malignant epithelial cells further raises a potential for inducing aberrant mutational activity (7, 8), yet the Ig protein of different isotypes expressed or secreted by cancer cells are also capable of favoring tumor growth.

Aberrant Igκ gene is shown to have transforming activity, which has been identified by transferring into non-transformed promotion-sensitive mouse epithelial cell line JB6(P+) cells, which could then be cloned in soft agar and led to tumor formation in nude mice (1).

Moreover, cancer cell-originated IgA protein plays a role in cellular transformation, too. We used mouse anti-human IgA to inhibit cancer-derived IgA. By MTT assay and growth curve analysis, we found that anti-human IgA could partly inhibit cellular transformation, promote the malignant proliferation ability of cancer cells, and increase the access percentage of S phase from the early mitosis of synchronized cancer cells. It was also demonstrated that blockade of cancer-derived Igα suppressed the growth and viability of cancer cells (31).

Qiu et al. got similar results for cancer-derived IgG. They demonstrated that cancer cells secreted IgG with unidentified specificity to promote growth and survival of tumor cells. Blockade of tumor-derived IgG by either antisense DNA or antihuman IgG antibody increased programmed cell death and inhibited growth of cancer cells *in vitro*; administration of antihuman IgG antibody also suppressed the growth of an IgG-secreting cancer line in immunodeficient nude mice, suggesting that cancer IgG expression is required, at least, in part, for survival of cancer cells (18).

These findings support the important role of cancer-derived Ig as a growth factor of cancer cells. It could be presumed that their function might be associated with some antibody related immunological courses and result in tumor evasion from immune surveillance.

Antibody-dependent cell-mediated cytotoxicity (ADCC) is a mechanism of cell-mediated immunity whereby an effector cell of the immune system actively lyses a target cell that has been bound by specific antibodies. In ADCC against tumor cells, antibody first binds *via* its antigen-binding site to its target on tumor cells, and then the Fc portion is recognized by specific Fc receptors on effector cells, stimulating their effector function by the release of cytotoxic granules, such as perforin, granulysin, and granzymes, whereas the release of cytokines and chemokines leads to

inhibition of cell proliferation and angiogenesis (32, 33). We analyzed the ADCC immuno-activity of Ig (including all isotypes) derived from cancer cells: cancer derived Ig was capable of reacting with FcR of monocytes, NK cells etc. by its Fc region as normal Ig, and to accomplish ADCC with effector cells (unpublished data). Based on these findings, it may be hypothesized that cancer-derived Ig could compete with B cell-derived Ig for the FcR on effector cells, thus inhibits ADCC and favors tumor immune escape.

Since Ig produced in cancer cell is closely related to cell malignancy, it is not surprising to find that the expression of Ig in tumor tissues is associated with increasing tumor grades. In order to avoid Ig endocytosis of epithelium cells, we used ISH (*in situ* hybridization) but not immunohistochemistry to observe the expression of Ig in cervical tissues of proceeding malignant grades. We detected a low level of mRNA for Igκ in epithelia with cervicitis. However, in epithelia with dysplasia and carcinoma, the expression of Igκ mRNA was markedly increased (34). A paralleled study got similar results for Igα too, in which only 2 subjects in 9 cervicitis are Igα mRNA positive, yet each one in 10 cervical carcinoma samples shows high level of Igα mRNA expressing (unpublished data). This characteristic pattern of cancer Ig expression may serve as a potential marker for malignant cell transformation.

Furthermore, by sequencing Igκ gene, two single nucleotide polymorphisms (SNP) locus rs232230 (5658C/G), rs232228 (3635T/C) were identified. The SNP 5658C/G is located in open reading frame on Igκ gene C region, resulted in amino acid change from Val to Leu. The SNP 3635T/C is located in the Matrix attached Region of Igκ gene. We hypothesize that it may influence gene rearrangement and regulation. By a case-control study, we found that the two SNPs were associated with susceptibility to nasopharyngeal carcinoma. The SNP 5658CG genotype was distributed higher in nasopharyngeal carcinoma patients than in normal individuals (35). We also identified that the two SNPs genotype frequency was associated with the susceptibility to gastric and breast cancer (unpublished data).

Some regulatory mechanisms of Ig expressed in cancer cells

In normal B cells, an important mechanism of activation and Ig production is CD40 ligation-triggered cellular signaling pathways. As a consequence of CD40 signaling, a number of well-characterized signal transduction pathways are activated, including the NF-κB, p38 mitogen-activated protein kinase, c-Jun-NH2-kinase, Janus kinases/signal transducers and activators of transcription, and phosphoinositide 3-kinase pathways. These pathways, in turn, regulate alterations in gene expression that are themselves extensive, dynamic, and variable, and finally induce proliferation and immunoglobulin class switching of resting B cells (36, 37).

Analogously, in cancer cells, the initiation and expression of Ig gene could be regulated by these cellular signaling pathways, too. Taking Igκ gene in NPC cell lines as a model, we may shed a light on the mechanism of cancer Ig expression regulation.

Igκ gene expression is under the control of distinct cis-regulatory elements, including the κ intron enhancer (iEκ) and the κ 3' enhancer (3'Eκ), which are located within the Jκ-Cκ region and downstream of Cκ region, respectively. The activation of enhancers is required for Igκ gene expression in B cell lines (38-40). The human Igκ gene contains an NF-κB site and a perfect consensus AP-1 site within the iEκ and the downstream iEκ. In B lymphocytes, both enhancers can be activated by nuclear factor NF-κB and AP-1 at different degrees (41, 42).

Epstein-Barr virus (EBV) encoded latent membrane protein 1 (LMP1) was the first EBV latent gene found to be able to transform cell lines and alter the phenotype of cells due to its oncogenic potential (43). LMP1 functions by resembling CD40 (44). It has been known to have oncogenic properties during latent infection in NPC. LMP1 alters many functional properties that are involved in tumor progression and invasions by activating its target genes *via* different signaling pathways (45-47).

In NPC cells, it has been noticed that the level of Igκ is significantly higher in LMP1-positive cells than in LMP1-negative cells, and LMP1 could increase the production of κ light chain at both mRNA and protein levels in a dose-dependent manner (48). Further investigation revealed that in LMP1-expressing NPC cells, Igκ gene enhancers iEκ and 3'Eκ are activated by binding with transcription factors NF-κB, AP-1 and Ets-1, all of which were modulated by LMP1-triggered pathways. It could be concluded that LMP1 stimulated transcription factors NF-κB, AP-1, Ets-1 binding to the corresponding site in Igκ gene *via* NF-κB, JNK/MAPK and ERK/MAPK signal pathways and finally upregulated Igκ light chain induction (49).

This presented novel experimental proofs on the mechanisms upregulating the ectopic expression of Igκ by LMP1 in NPC cells. Since other virus-encoded oncoproteins, such as HBX, E6, E7, can also activate many signal pathways including NF-κB, AP-1 and ERK pathways (50-52), these oncoproteins might induce Ig gene expression through the mechanism similar to EBV-LMP1. All these give us new insight into this abnormal phenomenon, which lays foundations for further studies, as well as new strategies for cancer therapy.

Owning to the heterogeneity characteristic of malignant cancer cells, the complexity of cancer tissue micro-environment and the close relationship between immune system and carcinogenesis, it is extremely difficult to clarify the specific mechanism and function of the abnormally expressed and secreted immunoglobulin in cancer cells. It is obvious that there are still a lot of blanks waiting to be filled in this field.

Acknowledgements

This work is supported by grants from China Medicine Board (No. 96655, No. 04-799), State Key Basic Research and

Development Plan (973) of the Ministry of Science and Technology of China (No. 2004CB518703), National High Technology Research and Development Program (863) of China (No. 2006AA02A404), Key Project of National Natural Science Foundation of China (No. 38930410, No. 30530710), National Natural Science Foundation of China (No. 39080015, No. 39400153, No. 39470299, No. 39600082, No. 69700170, No. 30171047, No. 30271218, No. 30471968, No. 30570700, No. 30772465), National Natural Science Funds for Distinguished Young Scholar (No. 39525022) and Key Project of Hunan Province (04SK1001).

References

1. Cao Y, Sun Y, Poirier S, et al. Isolation and partial characterization of a transformation-associated sequence from human nasopharyngeal carcinoma. Mol Carcinog. 1991;4:297-307.
2. Huber R. Spatial structure of immunoglobulin molecules. Klin Wochenschr. 1980;58:1217-1231.
3. Virella G, Wang AC. Immunoglobulin structure. Immunol Ser. 1993;58:75-90.
4. Bassing CH, Swat W, Alt FW. The mechanism and regulation of chromosomal V(D)J recombination. Cell. 2002;109 Suppl:S45-55.
5. Dudley DD, Chaudhuri J, Bassing CH, Alt FW. Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. Adv Immunol. 2005; 86:43-112.
6. Eckhardt LA. Immunoglobulin gene expression only in the right cells at the right time. FASEB J. 1992;6:2553-2560.
7. Okazaki IM, Hiai H, Kakazu N, et al. Constitutive expression of AID leads to tumorigenesis. J Exp Med. 2003;197:1173-1181.
8. Okazaki IM, Kotani A, Honjo T. Role of AID in tumorigenesis. Adv Immunol. 2007;94:245-273.
9. Gonzalez D, van der Burg M, Garcia-Sanz R, et al. Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. Blood. 2007;110:3112-3121.
10. Edry E, Melamed D. Class switch recombination: a friend and a foe. Clin Immunol. 2007;123:244-251.
11. Lefranc G, Lefranc MP. Regulation of the immunoglobulin gene transcription. Biochimie. 1990;72:7-17.
12. Li M, Tang M, Deng X. Positive immunoglobulin A expression in human epithelial carcinoma cell lines. Zhonghua Zhong Liu Za Zhi. 2001;23:451-453.
13. Kimoto Y. Expression of heavy-chain constant region of immunoglobulin and T-cell receptor gene transcripts in human non-hematopoietic tumor cell lines. Genes Chromosomes Cancer. 1998;22:83-86.
14. Li J, Tan C, Xiang Q, et al. Proteomic detection of changes in protein synthesis induced by NGX6 transfected in human nasopharyngeal carcinoma cells. J Protein Chem. 2001;20:265-271.
15. Okabe H, Satoh S, Kato T, et al. Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. Cancer Res. 2001;61:2129-2137.
16. Zheng S, Cao J, Geng L. Structure and expression of colorectal cancer related immunoglobulin novel gene SNC73. Zhonghua Yi Xue Za Zhi. 2001;81:485-488.
17. Hu JB, Zheng S, Deng YC. Expression of a novel immunoglobulin gene SNC73 in human cancer and non-cancerous tissues. World J Gastroenterol. 2003;9:1054- 1057.
18. Qiu X, Zhu X, Zhang L, et al. Human epithelial cancers secrete immunoglobulin G with unidentified specificity to promote growth and survival of tumor cells. Cancer Res. 2003;63:6488-6495.
19. Chen Z, Gu J. Immunoglobulin G expression in carcinomas and cancer cell lines. FASEB J. 2007;21:2931-2938.
20. Geng LY, Shi ZZ, Dong Q, et al. Expression of SNC73, a transcript of the immunoglobulin α-1 gene, in human epithelial carcinomas. World J Gastroenterol. 2007;13:2305- 2311.
21. Zhu X, Li C, Sun X, et al. Immunoglobulin mRNA and protein expression in human oral epithelial tumor cells. Appl Immunohistochem Mol Morphol. 2008;16:232-238.
22. Huang J, Sun X, Mao Y, et al. Expression of immunoglobulin gene with classical V-(D)-J rearrangement in mouse brain neurons. Int J Biochem Cell Biol. 2008;40:1604-1615.
23. Zheng H, Li M, Ren W, et al. Expression and secretion of immunoglobulin α heavy chain with diverse VDJ recombinations by human epithelial cancer cells. Mol Immunol. 2007; 44:2221-2227.
24. Xiao T, Ying W, Li L, et al. An approach to studying lung cancer-related proteins in human blood. Mol Cell Proteomics. 2005;4:1480-1486.
25. Ouyang G, Chen Y, Ruan L, et al. Differential phosphoproteome analysis of nasopharyngeal carcinoma and normal nasopharyngeal epithelial tissues. Int J Path Clin Med. 2007;27:461-469.
26. Nie G, Li M, Zhang P, et al. Differential proteomic analysis of sera from lung squamous carcinoma patients and healthy subjects. Int J Path Clin Med. 2007;27:470-475.
27. Goebel P, Janney N, Valenzuela JR, Romanow WJ, Murre C, Feeney AJ. Localized gene-specific induction of accessibility to V(D)J recombination induced by E2A and early B cell factor in nonlymphoid cells. J Exp Med. 2001;194:645-656.
28. Casali P, Schettino EW. Structure and function of natural antibodies. Curr Top Microbiol Immunol. 1996;210:167-179.
29. VanDyk L, Meek K. Assembly of IgH CDR3: mechanism, regulation, and influence on antibody diversity. Int Rev Immunol. 1992;8:123-133.
30. Babbage G, Ottensmeier CH, Blaydes J, Stevenson FK, Sahota SS. Immunoglobulin heavy chain locus events and expression of activation-induced cytidine deaminase in epithelial breast cancer cell lines. Cancer Res. 2006;66:3996-4000.
31. Zheng H, Li M, Liu H, et al. Immunoglobulin α heavy chain derived from human epithelial cancer cells promotes the access of S phase and growth of cancer cells. Cell Biol Int. 2007;31: 82-87.
32. Iannello A, Ahmad A. Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies. Cancer Metastasis Rev. 2005;24:487-499.
33. Weiner GJ. Monoclonal antibody mechanisms of action in cancer. Immunol Res. 2007;39:271-278.
34. Li M, Feng DY, Ren W, et al. Expression of immunoglobulin kappa light chain constant region in abnormal human cervical epithelial cells. Int J Biochem Cell Biol. 2004;36:2250-2257.
35. Ren W, Zheng H, Li M, et al. A functional single nucleotide polymorphism site detected in nasopharyngeal carcinoma-associated transforming gene Tx. Cancer Genet Cytogenet. 2005;157:49-52.
36. Vonderheide RH. Prospect of targeting the CD40 pathway for cancer therapy. Clin Cancer Res. 2007;13:1083-1088.
37. Xu Y, Song G. The role of CD40-CD154 interaction in cell

immunoregulation. J Biomed Sci. 2004;11:426-438.
38. Pongubala JM, Atchison ML. Activating transcription factor 1 and cyclic AMP response element modulator can modulate the activity of the immunoglobulin kappa 3' enhancer. J Biol Chem. 1995;270:10304-10313.
39. Inlay MA, Gao HH, Odegard VH, Lin T, Schatz DG, Xu Y. Roles of the Igκ light chain intronic and 3' enhancers in Igκ somatic hypermutation. J Immunol. 2006;177:1146-1151.
40. Inlay MA, Lin T, Gao HH, Xu Y. Critical roles of the immunoglobulin intronic enhancers in maintaining the sequential rearrangement of IgH and Igκ loci. J Exp Med. 2006;203:1721-1732.
41. Schanke JT, Marcuzzi A, Podzorski RP, Van Ness B. An AP1 binding site upstream of the κ immunoglobulin intron enhancer binds inducible factors and contributes to expression. Nucleic Acids Res. 1994;22:5425-5432.
42. Schanke JT, Van Ness BG. Organization of the transcription factor binding sites in the κ Ig intron enhancer. Effects of position, orientation, and spacing. J Immunol. 1994;153:4565-4572.
43. Izumi KM. Epstein-Barr virus signal transduction and B-lymphocyte growth transformation. Prog Mol Subcell Biol. 2004;36:269-288.
44. Lam N, Sugden B. CD40 and its viral mimic, LMP1: similar means to different ends. Cell Signal. 2003;15:9-16.
45. Zheng H, Li LL, Hu DS, Deng XY, Cao Y. Role of Epstein-Barr virus encoded latent membrane protein 1 in the carcinogenesis
46. of nasopharyngeal carcinoma. Cell Mol Immunol. 2007;4:185-196.
47. Burgos JS. Involvement of the Epstein-Barr virus in the nasopharyngeal carcinoma pathogenesis. Med Oncol. 2005;22:113-121.
48. Eliopoulos AG, Young LS. LMP1 structure and signal transduction. Semin Cancer Biol. 2001;11:435-444.
49. Liao W, Tang M, Li JJ, Leo ML, Cao Y. Epstein-Barr virus encoded latent membrane protein 1 increases expression of immunoglobulin κ light chain through NFκB in a nasopharyngeal carcinoma cell line. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao. 1999;31:659-663.
50. Liu HD, Zheng H, Li M, Hu DS, Tang M, Cao Y. Upregulated expression of κ light chain by Epstein-Barr virus encoded latent membrane protein 1 in nasopharyngeal carcinoma cells *via* NF-κB and AP-1 pathways. Cell Signal. 2007;19:419-427.
51. Chan CF, Yau TO, Jin DY, Wong CM, Fan ST, Ng IO. Evaluation of nuclear factor-κB, urokinase-type plasminogen activator, and HBx and their clinicopathological significance in hepatocellular carcinoma. Clin Cancer Res. 2004;10:4140-4149.
52. Chung TW, Lee YC, Kim CH. Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential. FASEB J. 2004;18:1123-1125.
53. Tommasino M, Crawford L. Human papillomavirus E6 and E7: proteins which deregulate the cell cycle. Bioessays. 1995;17:509-518.