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BRIEF COMMUNICATION

Expression of Heavy-Chain Constant Region of Immunoglobulin and T-Cell Receptor Gene Transcripts in Human Non-Hematopoietic Tumor Cell Lines

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Expression of gene transcripts for immunoglobulins and a T-cell receptor was investigated in non-hematopoietic tumor cell lines using the highly sensitive RT-nested PCR method. These proteins are reported to be produced and secreted or expressed in malignancies originating from hematopoietic organs only. Originally designed PCR primers for different exons coding for the heavy-chain constant regions of IgM, IgD, IgG3, IgG1, IgE, and IgA and the T-cell receptor-α were used. All gene transcripts were detected in the 5 investigated cancer cell lines without exception. The results suggest that even non-hematopoietic cancer cells transcribe immunoglobulin and T-cell receptor genes and may produce the corresponding proteins. *Genes Chromosomes Cancer 22:83–86, 1998.*

Immunoglobulins (Ig) are proteins produced and secreted by differentiated B lymphocytes. The T-cell receptor (TCR) is a membrane-bound protein expressed on T cells or related lymphocytes. The human malignant neoplasm that produces immunoglobulins is malignant myeloma. TCR is reported to be expressed in adult T-cell leukemia. There is no evidence that other malignant tumor cells, especially cancer cells, produce immunoglobulins or TCR protein; the genes encoding these proteins are believed not to be transcribed.

In this study, the highly sensitive polymerase chain reaction (PCR) method in which nested PCR was repeated several times was applied to investigate the possible expression of extremely small amounts of mRNA coding for the heavy-chain constant region of IgM, IgD, IgG3, IgG1, IgE, and IgA as well as the TCR-α in human carcinoma cell lines. The human malignant cell lines investigated were as follows: 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, none of which is known to secrete any immunoglobulins. The cell lines were cloned and the absence of lymphocyte contamination was confirmed microscopically before RNA preparation. mRNA from 10^4 tumor cells was collected by the acid guanidinium-phenol-chloroform method (Isogen, Nippon gene) and was reverse-transcribed with a reverse transcription mixture containing 1.2 μM of random hexamer, 5.6 U of RNasin (Promega, Madison, WI) and 80 U of M-MLV reverse transcriptase (Gibco BRL, Gaithersburg, MD). Twenty microliters of PCR mixture contained 1 μM of sense and antisense primers and 1 U of *Taq* DNA polymerase (Takara Taq). Two microliters of each PCR product were used in the following reaction. Usually 3 sense and 3 or 4 antisense primers were combined to reach 5 PCR reactions in nested or heminested fashion. All nested or heminested PCR experiments were performed using 30 cycles of 94°C 40 sec, 62°C 1 min, and 72°C 1 min. Originally designed PCR primers used in this study were: IgM (Word et al., 1989; accession number: X57331): sense, 5′-GGCGAAGCTGTGAAAACCCACACC-3′ [1268-1291], 5′-GAGGCCAGCATCTGCGAGGATGAC-3′ [1337-1360], 5′-CGGGGAGAGGTTCACGTGCACCG-3′ [1369-1391]; antisense, 5′-CTCAGGCATTGGGGCGCTGGTCAC-3′ [1811-1788], 5′-CCACTGCACGAAGACGTCCGCGGG-3′ [1751-1728], 5′-GCCGACTCCCGCAGGTTCAGCTGC-3′ [1696-1673]. IgD (Word et al., 1989; accession number: X57331): sense, 5′-CACCTGACCTGGGAGGTGGCTGGG-3′ [15316-15339], 5′-GCTGCTGGAGCGGCACAGCAACGGC-3′ [15369-15393],

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TABLE 1. Investigated mRNA

Protein	Gene	Map location	Size of PCR product (bp)		Primer site (exon)
			1st PCR (invisible)	Final PCR (visible)	
IgM	*IGHM*	14q32.33	364	148	Cμ3–Cμ4
IgD	*IGHD*	14q32.33	269	134	Cδ2–Cδ3
IgG3	*IGHG3*	14q32.33	263	194	Ch1–Ch4-C
IgG1	*IGHG1*	14q32.33	122	71	CH1-hinge-CH2
IgE	*IGHE*	14q32.33	506	181	CH2–CH4
IgA	*IGHA*	14q32.33	508	262	1–3
TCRα	*TRA*	14q11.2	279	153	1–3

5′-GAGCCAGCACAGCCGTCTGACCC-3′ [15399-15421]; antisense, 5′-CAGGAGCCACGAGGCCGCCTCGGG-3′ [15803-15780], 5′-CAGACGAGGCCAGCAGGTTCAGGG-3′ [15774-15751], 5′-CGGGTGCCTGCGCAGCGGGTTCTC-3′ [15741-15727, 15517-15509]. IgG3 (Huck et al., 1986; accession number: X04646): sense, 5′-GCAACACCAAGGTGGACAAGAGAG-3′ [9-32], 5′-GGTGGACAAGAGAGTTGAGCTC-3′ [19-35, 427-431], 5′-GCTCAAAACCCCACTTGGTGACAC-3′ [428-451]; antisense, 5′-GGGTTTTGGGGGGAAGAGGAAGAC-3′ [1210-1186], 5′-GAGGAAGACTGACGGTCCTCCCAG-3′ [1194-1171], 5′-GTTCAGGTGCTGGGCACCTTGGGC-3′ [1168-1160, 1041-1027]. IgG1 (Walls et al., 1993; accession number: Z17370): sense, 5′-GCAACACCAAGGTGGACAAGAGAG-3′ [479-502], 5′-GGTGGACAAGAGAGTTGAGCTC-3′ [488-505, 897-901]; antisense, 5′-GGGTTTTGGGGGGAAGAGGAAGAC-3′ [1109-1086], 5′-GAGGAAGACTGACGGTCCTCCCAG-3′ [1094-1071], 5′-GTTCAGGTGCTGGGCACCTTGGGC-3′ [1068-1060, 941-927]. Two sense primers and 3 antisense primers for the detection of IgG1 mRNA were the same as those for IgG3. The PCR products detected by these 5 primers were 71 base pairs and the sequence coincided with that of the mRNA for the IgG1 constant region. IgE (Flanagan and Rabbitts, 1982; Kenten et al., 1982; accession number: L0022): sense, 5′-GGACGTGGACTTGTCCACCGCCTC-3′ [771-794], 5′-GCACTGGCTGTCAGACCGCACCTAC-3′ [852-876], 5′-GAAGTGTGCAGATTCCAACCCGAG-3′ [924-934, 1021-1033]; antisense, 5′-CCGGGGCAGCACGGCGGGCCGCTGG-3′ [1445-1428, 1344-1339], 5′-CGATCCAGTCTCGGGTGCCCACCG-3′ [1266-1243], 5′-GGTGGAGTGGTTCACAGGCTTCCC-3′ [1190-1167]. IgA(α1) (Flanagan et al., 1984; accession number: K01312): sense, 5′-GGGGACCTGTACACCACGAGCAGC-3′ [321-344], 5′-GCCGGCAAGTCCGTGACAGTCCAC-3′ [375-398], 5′-GGATGTGACTGTGCCCTGCCCAG-3′ [425-447]; antisense, 5′-CCTCGGGCCGGAATGTGTTTCCGG-3′ [1264-1243, 1021-1019], 5′-GGGTGGCGGTTAGCGGGGTCTTGG-3′ [1009-986], 5′-CGGCACAGCCCGGCAGGACAC TGG-3′ [934-911], 5′-GCAGCCACAGAGGTCACGCTCAGG-3 [900-877]. TCR-α (Rabbitts et al., 1985; accession number: X02592): sense, 5′-GTGCTAGACATGAGGTCTATGGAC-3′ [681-704], 5′-GCAACAGTGCTGTGGCCTGGAGC-3′ [712-734], 5′-GCATGTGCAAACGCCTTCAACAACAGC-3′ [750-776]; antisense, 5′-GCTGGACCACAGCCGCAGCGTC-3′ [959-938], 5′-GCAGATTAAACCCGGCCACTTTCAGG-3′ [933-908], 5′-GGATTCGGAAGGGAATCACTGACAGG-3′ [903-878]. The genes encoding the constant regions of these immunoglobulins and of TCR-α possess plural exons, and in order to detect mRNA, primers were designed for different exons. Investigation according to Sanger's method revealed that the final PCR products visible on acrylamide gel had intronless correct sequences. The name and map location of the genes, size of the 1st and final visible 5th PCR products, and the exon numbers where PCR primers were located are given in Table 1. Two microliters of the PCR mixture was electrophoresed (constant voltage, 150 V) on an acrylamide gel (8%) that was stained with ethidium bromide. Since the amounts of the PCR products were less than that detectable by ethidium bromide, the PCR products except the final one were invisible on acrylamide gel.

As shown in Figure 1, heavy-chain constant regions for IgM, IgD, IgG3, IgG1, IgE, and IgA and TCR-α mRNA were all detected in all 5 tumor cell lines. After these studies pure water and mRNAs of chicken splenocytes, fish *medaka*, and leaf of chickweed were investigated as negative controls, but aimed PCR products were never detected. Expression of, for example, IgD mRNA, which could most easily be detected in human lymphocytes, was not detected in other species nor in pure water (Fig. 2).

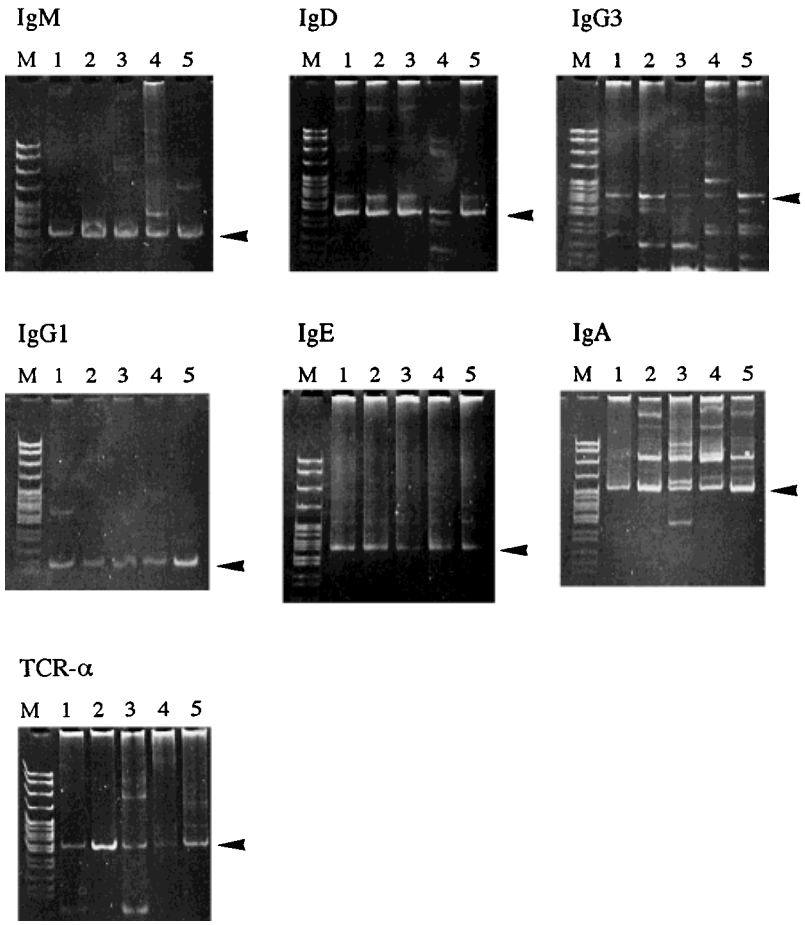

Figure 1. Expression of heavy-chain constant region of immunoglobulin and T-cell receptor gene transcripts in human cancer cells. M: pBR322/Msp I digest; 1: SW1116; 2: HEp2; 3: MCF-7; 4: MDA-MB-231; 5: HC48.

The result suggests that the constant regions of immunoglobulins and TCR-α genes were transcribed even in non-hematopoietic tumor cells. In lymphocytes, sterile transcripts, which are initiated from promoters just upstream of the constant region genes, are expressed in the absence of DNA rearrangement, and possibly these non-hematopoietic cancer cell lines too possess such sterile transcripts of immunoglobulins and TCR constant region genes. However, another hypothesis may also be presented. There is no evidence that DNA rearrangement or class switch of immunoglobulins occurs in cancer cells. Therefore, the mRNA detected in this study could be transcribed spontaneously either from one gene or from both genes without any functional or differentiational meaning. Even though these mRNAs were translated to corresponding proteins, the constant regions of immunoglobulins and TCR-α would not be functional unless DNA rearrangement of the variable regions occurred.

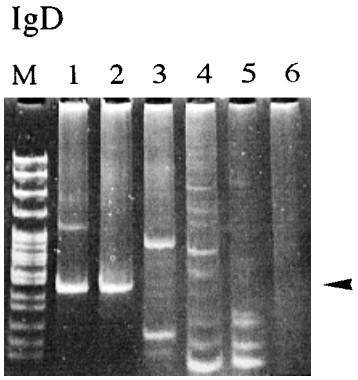

Figure 2. Expression of IgD constant region mRNA in human lymphocytes but not in chicken splenocytes, a fish *medaka,* or leaf of chickweed. M: pBR322/Msp I digest; 1: human lymphocytes; 2: interleukin 2-activated human lymphocytes; 3: chicken splenocytes; 4: whole body of a fish *medaka;* 5: leaf of chickweed; 6: H_2O.

This study revealed that even non-hematopoietic cancer cells express mRNA for immunoglobulins and TCR, but probably without complete

function. These gene transcripts were also detected in a human single sperm and a single lymphocyte (Kimoto, in press). This fact supports the hypothesis that all gene transcripts exist in every cell: all mRNAs are preserved in a human single cell (Kimoto, 1996). Moreover, gene transcripts for cerebral and digestive hormones, hormone receptors, surface molecules, and cytokines were expressed in all investigated tumor cell lines or normal cells (Kimoto, 1995). There are some reports on ectopic expression of mRNA or ectopic production of proteins (Humphries et al., 1976; Weintraub and Groudine, 1976; Chelly et al., 1988, 1989). It can be concluded that all transcribable genes are transcribed in every cell. These facts also suggest that tumors can be changed to normal cells when the mechanism for gene transcription is completely understood.

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REFERENCES

Chelly J, Kaplan JC, Maire P, Gantron S, Kahn A (1988) Transcription of the dystrophin gene in human muscle and non-muscle tissues. Nature 333:858–860.

Chelly J, Concordet JP, Kaplan JC, Kahn A (1989) Illegitimate transcription: Transcription of any gene in any cell type. Proc Natl Acad Sci USA 86:2617–2621.

Flanagan JG, Rabbitts TH (1982) The sequence of a human immunoglobulin epsilon heavy chain constant region gene, and evidence for three non-allelic genes. EMBO J 1:655–660.

Flanagan JG, Lefranc MP, Rabbitts TH (1984) Mechanisms of divergence and convergence of the human immunoglobulin α1 and α2 constant region gene sequences. Cell 36:681–688.

Huck S, Keyeux G, Ghanem N, Lefranc MP, Lefranc G (1986) A gamma 3 hinge region probe: First specific human immunoglobulin subclass probe. FEBS Lett 208:221–230.

Humphries S, Windass J, Williamson R (1976) Mouse globin gene expression in erythroid and non-erythroid tissues. Cell 7:267–277.

Kenten JH, Molgaard HV, Houghton M, Derbyshire RB, Viney J, Bell LO, Gould HJ (1982) Cloning and sequence determination of the gene for the human immunoglobulin epsilon chain expressed in a myelomas cell line. Proc Natl Acad Sci USA 79:6661–6665.

Kimoto Y (1995) Gene transcripts of eleven proteins with specific functions are all detected in human normal cells and tumor cell lines: A possible DNA → RNA basal constant flow. Hum Cell 8:202–210.

Kimoto Y (1996) A possibility of all mRNA expression in a human single lymphocyte. Hum Cell 9:367–370.

Kimoto Y A human single cell expresses all messenger ribonucleic acids: The arrow of time in a cell. Mol Gen Genet (in press).

Rabbitts TH, Lefranc MP, Stinson MA, Sims JE, Schroder J, Steinmets M, Spurr NL, Solomon E, Goodfellow PN (1985) The chromosomal location of T-cell receptor genes and a T cell rearranging gene: Possible correlation with specific translocations in human T cell leukaemia. EMBO J 4:1461–1465.

Walls MA, Hsiao KC, Harris LJ (1993) Vectors for the expression of PCR-amplified immunoglobulin variable domains with human constant regions. Nucleic Acids Res 21:2921–2929.

Weintraub H, Groudine M (1976) Chromosomal subunits in active genes have an altered conformation. Science 193:848–856.

Word CJ, White MB, Kuziel WA, Shen AL, Blattner FR, Tucker PW (1989) The human immunoglobulin C μ-C delta locus: Complete nucleotide sequence and structural analysis. Int Immunol 1:296–309.