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Molecular analysis of the IL-6 receptor in human multiple myeloma, an IL-6-related disease

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Abstract

A PCR-SSCP approach was used to search for mutations in IL-6 receptor genes in 9 human plasma cell lines (HMCL) and in tumor plasma cells from 19 patients with fulminating multiple myeloma, an IL-6-related disease. Whereas no mutation was found in the cytokine receptor homologous (CRH) domain of IL-6Rα, DNA and RNA polymorphisms in the gp130 CRH domain was detected in tumoral samples as well as in blood samples from healthy donors. Finally, mutations in the gp130 critical cytoplasmic domain were found in one HMCL and in tumor plasma cells of one patient. Only the mutated allele was expressed in the HMCL.

Key words: Multiple myeloma; IL-6; PCR-SSCP; Cytokine

1. Introduction

Human multiple myeloma (MM) is a B-cell neoplasia affecting the late stage of B-cell differentiation, i.e. the plasma cell. In this disease, interleukin (IL)-6 has been shown to be a major growth factor in vitro and in vivo [1–3]. Initially, IL-6 was considered to be an autocrine growth factor produced by tumor cells themselves [1,4,5], suggesting that deregulated IL-6 gene expression could be the major oncogenic event in this disease. However, most groups now agree that the autocrine hypothesis is limited to only a few myeloma cell lines cultured for many years in vitro [6–8], and that, in vivo, IL-6 is produced by the tumor environment (bone marrow stromal cells [9]), possibly as a result of abnormalities in these stromal cells [10]. Thus, high levels of IL-6 circulate in patients with MM in vivo [11], the IL-6 receptor is present in various normal cells in vivo and IL-6 is a growth factor of these cells [12] (dermal cells, myeloid cells, B cells, ...). However only the tumoral clone emerges by using this cytokine as a growth factor. Hypothesis for explaining the IL-6 dependent emergence of the tumoral clone in vivo is an abnormal signal mediated by IL-6 receptor activation. These observations suggest that the IL-6 receptor and/or the IL-6 receptor-mediated transduction signal, and/or the function induced by this transduction signal (proliferation/differentiation), are targets of the oncogenic process. The IL-6R belongs to the hemopoietic growth factor receptor family [13–15], and is typically made by the association of two chains (the IL-6 binding chain, IL-6Rα and the IL-6 signal-transducing chain, gp130) which form the active receptor. This family of receptors is characterized by a 200 amino acid extracytoplasmic domain referred to as the cytokine receptorhomologous (CRH) domain [15]. Previous studies using mutagenesis showed the major role, for receptors of IL-2, IL-6, erythropoietin (Epo) and growth-hormone (GH), of the CRH domain in binding and response to their respective ligands [16–21]. Yawata et al. (1993) demonstrated that the IL-6Rα CRH domain is responsible both for the binding of the cytokine and the association with gp130 to generate the IL-6 signal. For two members of this family, mutations in the CRH domain have been shown to be associated with abnormal response to growth factors: substitution of any of five amino acids for an alanine residue in the CRH domain of human growth hormone receptor slightly increases affinity for the ligand [21]; erythropoietin receptor (EpoR) can be constitutively activated by a single point mutation in the CRH domain, converting the residue R to C at position 129 [19]. This was further supported by transfection experiments where the mutated Epo-R was introduced into Ba/F3 cells, which were subsequently

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Abbreviations: MM, multiple myeloma; PCL, plasma cell leukemia; BM, bone marrow; PB, peripheral blood; PE, pleural effusion; HMCL, human myeloma cell line; CRH, cytokine receptor homologous domain; IL, interleukin; Epo, erythropoietin; GH, growth hormone; RT, reverse transcript; PCR, polymerase chain reaction; SSCP, single strand conformational polymorphism; aa, amino acid.

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rendered tumorigenic [19]. Moreover, mice infected with a recombinant spleen focus-forming retrovirus, in which the env gene is replaced by the EpoR cDNA mutated at codon 129, develop erythroleukemia [22].

In addition to the CRH domain, the transducing chains of this hematopoietic growth factor family show homologies in a cytoplasmic region proximal to the transmembrane domain [23–25]. The gp130 first 61 intracytoplasmic amino acids corresponding to this homologous region are sufficient to generate the growth signal [24]. In contrast to gp130, IL-6Rα, though lacking both transmembrane and intracytoplasmic domains, is still functional. Soluble IL-6Rα, corresponding only to the extracytoplasmic domain, can associate with gp130 in the presence of IL-6 and mediate its function [26]. Accordingly, we investigated the IL-6Rα and gp130 CRH domains and the 61 cytoplasmic amino acids of gp130. We analyzed RNA or DNA using the PCR-SSCP method alone or in combination with reverse transcription (RT-PCR-SSCP) and studied variant sequences by a direct sequencing technique.

2. Material and methods

2.1. Cell lines

Nine human myeloma cell lines (HMCL) were studied. RPMI 8226 and U266 HMCL are autonomously growing HMCL obtained 20 years ago and purchased by us from the ATCC. The other 7 HMCL, termed XG-1 to XG-7, were obtained in our laboratory from patients with terminal disease and extramedullary proliferation [27]. XG-1 to XG-7 HMCL are dependent on the addition of IL-6 for in vitro growth. Three EBV-infected B-cell lines (EBV-BCL) were used as controls: the CESS cell line purchased from ATCC and two other cell lines obtained in our laboratory after infection by EBV of peripheral blood lymphocytes from healthy donors.

2.2. Freshly-explanted tumor samples

Tumoral samples were obtained after informed consent. Eight tumoral samples were obtained from bone marrow of 8 patients with medullary involvement only, 9 from peripheral blood of patients with plasma cell leukemia and 2 from pleural effusions. All patients with MM had an active disease, and all those with extramedullary proliferation were in a terminal stage. 12/19 of these samples contained more than 50% of plasma cells. For the others samples, plasma cells represented at least 10% of the population. Mononuclear cells were harvested by Ficoll-Hypaque density and kept in liquid nitrogen until use. Some of these samples were previously analyzed for P53 and Ras mutations [28,29].

2.3. Control samples

Peripheral blood mononuclear cells from 27 healthy donors, obtained after informed consent, were analyzed. Mononuclear cell fractions were separated by Ficoll-Hypaque density gradient centrifugation and kept in liquid nitrogen until needed.

2.4. Primers

Primers used for amplification of IL-6R CRH domain were: aggtcggtgcagctccacgac (sense) and gacacctcgttctcagctg (antisense) for the first step of nested PCR, then gtgcacttgctggtggatg (sense), ggccggactgttctgaaac (antisense); ggctgtgctcttggtgagg (sense), cccgacactactggcgacgca (antisense); tgcgtcgccagtagtgtcggg (sense), gttccaggagtgggggtcttgc (antisense); cccgctggctcagtgtcacctg (sense), ggccgctccaggcgtcgtgg (antisense); cacaacatggatggtcaagg (sense), gattctgtccaaggcgtg (antisense). The gp130 CRH domain was amplified by using the primers cagaacagcatccagtgtcacc (sense) and ccagaaacttggtgctttagatgg (antisense) for the first step of nested PCR, then cttacattcggacagcttgaacag (sense), cttgtgtgttgcccattcag (antisense); ggaagggaaacacacttggag (sense), gggcttcactttatatacagg (antisense); cctgtatataaagtgaagccc (sense), ggatgctgtgtcttcaggagg (antisense); cctcctgaagacacagcatcc (sense), cataggtgatcccacttgc (antisense). And, gp130 critical cytoplasmic domain was amplified by using the primers cttctgggagtgctgttctgc (sense), ccacaacacttacatcagtg (antisense); ctcacactcctccaaggcac (sense), caataccactgctgtgtccttc (antisense).

2.5. RNA preparation and cDNA synthesis

Frozen cells were disrupted in guanidium thiocyanate solution (4 M guanidium thiocyanate; 25 mM trisodium citrate, pH 7; 0.5% sarcosyl; 0.1 M 2-mercaptoethanol) by drawing the lysate through a 23-gauge needle. RNA was pelleted by ultracentrifugation of the homogenate over a cesium chloride gradient (5.7 M CsCl, 100 mM EDTA). The pellet was ethanol-precipitated with 3 M sodium acetate and resuspended in water. cDNA was synthesized with 2 μg of total cellular RNA and 500 ng of oligo(dT) primer using the Reverse Transcription System PCR-related kit (Promega) according to the manufacturer's recommendations.

2.6. DNA extraction

Cells were lysed in 0.3 M lithium acetate, 1 mM EDTA (pH 8), 10 mM Tris pH 8 and 2% SDS. DNA was phenol/chloroform-extracted and ethanol-precipitated before being resuspended in 10 mM Tris, 1 mM EDTA (pH 8).

2.7. PCR amplification

Nested oligonucleotide amplification was performed for CRH domain analysis. Two μl of reverse transcription products were initially amplified for 30 cycles in a final volume of 25 μl containing 10 mM Tris-HCl (pH 8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 100 μM dNTPs, 20 pmol of each primer and 1.5 U of Taq polymerase (Perkin-Elmer Cetus). A 2 μl aliquot of this amplified mixture was used for a second round of PCR (30 cycles), with oligonucleotide primers internal to the original primers, in the same conditions plus 3 μCi of [α-^{32}P]dCTP.

For intracytoplasmic domain analysis, 2 μl of reverse transcription products or 100 ng of DNA were amplified in the same conditions with 3 μCi of [α-^{32}P]dCTP.

2.8. SSCP analysis

An aliquot of the radiolabeled amplification products was diluted fivefold in 0.1% SDS, 10 mM EDTA. Four μl of this solution was then mixed with 7 μl of 95% formamide, 10 mM EDTA, Bromophenol blue and xylene cyanol, heated at 90°C for 3 min, chilled on ice and loaded (4 μl/lane) onto a nondenaturing polyacrylamide gel (6% acrylamide, 1 × TBE). Electrophoresis was performed in a cold room at 18 W for 7–8 h. Alternatively, 5% glycerol was added to the gel mix and electrophoresis was carried out at room temperature. The gels were exposed to Kodak XAR-5 film at −80°C using an intensifying screen.

2.9. Direct sequencing

Shifted SSCP bands were excised from the gel and eluted in 50 μl of sterile water for 2 h at 65°C. Four μl of the eluate was subjected to a further round of PCR amplification, then washed using a Centricon 30 column (Amicon) and sequenced using the Sequenase version 2.0 kit (USB). Conditions were according to the manufacturer's recommendations. Sequences were determined on both strands.

3. Results

3.1. Lack of mutation in the IL-6Rα CRH domain

A SSCP analysis can detect mutations in DNA fragments of less than 200 bp, the 630 bp IL-6R CRH domain was subdivided using 5 overlapping primer pairs. With the RT-PCR-SSCP approach, mutations were found in some samples but not reproducibly in different reverse-transcripts of the same RNA samples, which sug-

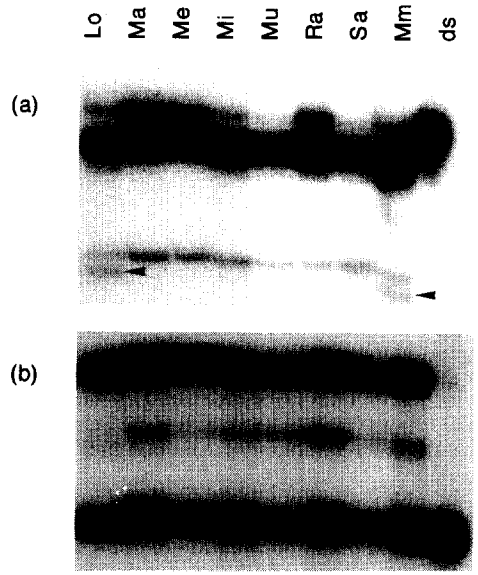

Fig. 1. PCR SSCP analysis of the gp130 CRH domain. Amplified RT fragments from aa 106 to 168 were denatured by heating, and electrophoresis was performed in 6% polyacrylamide gel at a constant 18 W. The right hand side lane shows double-strand migration. The arrowheads point to the aberrantly migrating fragments. (a) gel running in the cold room; (b) gel running at room temperature with 5% glycerol.

gest that these mutations were induced by reverse transcriptase. As all the mutations were found between amino acids (aa) 221 and 258, we chose a new primer pair enclosing this region and usable on DNA. With this approach, no mutations of the IL-6R CRH domain were identified in the 9 HMCL and 19 tumor samples analyzed.

3.2. *No mutation but sequence polymorphism in the gp130 CRH domain*

The gp130 CRH domain was analyzed by PCR-SSCP from RT-RNA. Nested PCR was used in this approach as well, and the CRH domain was subdivided using 4 overlapping primer pairs. Bands shifts were found only in the fragment spanning from aa 106 to 168. As illustrated in Fig. 1, two different mobility shifts were found upon SSCP analysis. Determination of the nucleotide sequence revealed that these two mobility band shifts were due to a substitution leading to a non-conservative amino acid change at position 148, replacing Gly with Arg (GGT to CGT) in the first case (Fig. 2a), and to a conservative A to G transition within codon 122 for the second shift (Fig. 2b). Substitution 148 was found in 5 of the 9 HMCL and 6 of the 19 MM samples analyzed. Substitution 122 was found in 1 HMCL and 5 MM samples. The systematic nature of these changes suggested to us that they could in fact be due to a sequence polymorphism. In order to prove that these substitutions were indeed the result of DNA polymorphism, we studied 3 EBV-infected B-cell lines and 27 healthy donors' peripheral blood lymphocytes were analyzed. This analysis revealed substitution 148 in one EBV cell line and in 9 healthy donors' peripheral blood lymphocytes, whereas 1 EBV cell line presented substitution 122. Interestingly in the population presently studied we did not detect any sample homozygous for the aa122 or 148 polymorphisms. This can most probably be related to the rather small size of the population studied and to the relative low frequency of these polymorphisms (6 and 18%, respectively, for the positions 122 and 148).

3.3. *Mutations in the intracytoplasmic domain of gp130*

The intracytoplasmic domain of gp130 was investigated from DNA since there is no intron in this region. The same samples were analyzed as for the gp130 CRH domain. Our aim was to determine whether there were mutations in the first 61 aa located closest to the transmembrane domain. The primers used covered the first 87 intracytoplasmic aa which were divided into two overlapping fragments. In this region, one HMCL (U266) and one myeloma sample showed band shifts (Fig. 3). The U266 cell line presented a C-to-T substitution at codon 710, converting Ser to Leu (Fig. 4a). This mutation affected the 69th intracytoplasmic aa and was thus downstream from the first 61 aa near the transmembrane domain. Patient Mi presented 5 mutations in this region: one conservative nucleotide substitution at codon 711

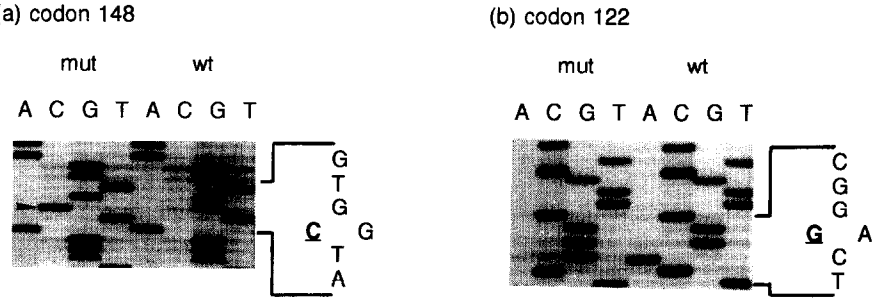

Fig. 2. gp130 polymorphism detected by PCR-amplified fragments. Each substitution shown is matched to a control RT ('mut' for mutated and 'wt' for wild type) The codon at which substitution occurs is indicated. Each sequence is shown 5′ (bottom) to 3′ (top). The nucleotide sites of mutation are indicated by underlined letters. Arrowheads point to bands corresponding to mutated bases. (a) sequence of band shift obtained from SSCP gel running in the cold room; (b) sequence of band shift obtained from SSCP gel running at room temperature with glycerol.

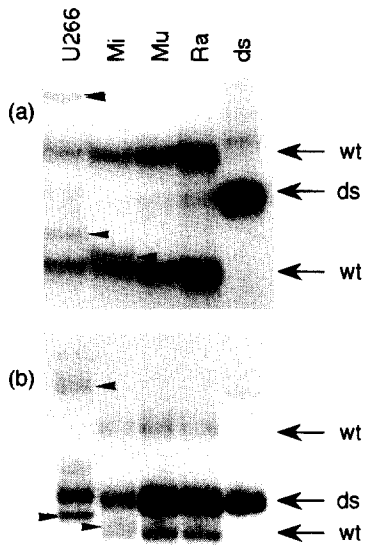

Fig. 3. SSCP analysis of the gp130 cytoplasmic domain. Amplified fragments from aa 668 to 729 were run on a 6% polyacrylamide gel in the cold room. Each lane is identified by the name of the sample analyzed, 'wt' (wild type) indicates a non-mutated control and 'ds' (double strand) corresponds to an undenatured sample. Arrowheads point to band shifts. (a) SSCP analysis from DNA; (b) SSCP analysis from RT.

(TTG to TTA) and 4 nonconservative substitutions converting Ser to Ala (TCA to GCA), Ser to Gly (AGT to GGT), Val to Phe (GTT to TTT) and Pro to Ala (CCA to GCA) at codons 678, 692, 693 and 705 respectively (Fig. 4b). U266, like the myeloma sample, presented the wild-type allelle and the mutated fragment. On the basis of these results, we analyzed this region from RT-RNA. As illustrated in Figure 3, the two alleles were expressed in patient Mi's bone marrow myeloma sample, while only the mutated allele was expressed in the U266 cell line. No mutations were found in DNA samples of the 3 EBV-infected B-cell lines and the 27 healthy donors' peripheral blood lymphocytes analyzed.

4. Discussion

In the present study we analyzed 18 MM and 9 HMCL by PCR-SSCP for the presence of point mutations in the genes coding IL-6Rα and gp130 chains. The rational for undertaking this is based on a series of converging observations: (i) the prominent role of IL-6 in the pathogenesis of MM, (ii) the oncogenic activation of the Epo-R by a nonconservative point mutation [19], (iii) the increased binding affinity of the mutated forms of the GH-R for GH [21], (iv) the directed mutagenesis study by Yawata and coworkers showing the essential role of the IL-6Rα CRH domain in conveying the IL-6 signal. We therefore considered it important to test for the presence of potentially activating point mutations in both chains of the IL-6 receptor, and given the fact that the known activating mutations all affected the CRH domain, we logically directed our first efforts at this region. Using total RNA as a template we analyzed by RT-PCR and SSCP a stretch of 638 bases divided between 5 overlapping amplimers. None of the 28 tumors samples demonstrated a reproducibly detectable mutated variant affecting this region of the gene. One might attribute this to a lack of sensitivity of the method. Although we cannot formally disprove this, we do not consider a technical problem could account for the present results. While analyzing this portion of the IL-6Rα gene, we detected mutated variants which upon verification corresponded to mutations introduced by either the reverse transcriptase or the Taq polymerase. We therefore considered these polymerase artifacts as positive controls for our assay. The lack of mutations in the IL-6Rα gene cannot be explained by the patient population studied. Indeed, all 19 patients had an active form of the disease, and 11 out of the 19 had terminal fulminant extramedullary disease, a stage of the disease in which frequent mutations of p53 and Ras genes are found [28–30]. Other IL-6R abnormalities in myeloma cells could involve deregulated expression of the protein. This was demonstrated by upregulated IL-6R gene expression achieved by insertion of intracisternal particles into the IL-6R gene in a murine plasmacytoma cell line [31]. Once the promoter and regulating regions of the IL-6R gene are known, it will be of interest to search for mutations in these regions in myeloma cells.

We also studied the gp130 CRH domain, the exact function of which has not been clearly determined. However, studies with other transducing chains of the haematopoietic receptor family (G-CSF-R, EPO-R, GH-R, etc.) [19–21,23] strongly suggest that this domain is essential for binding to the IL-6Rα/IL-6 complex and dimerization of the gp130 chain induced upon activation [32]. Interestingly, we detected polymorphism of the gp130 gene which was expressed at the RNA level both in patients with MM and healthy individuals. This polymorphism was due first of all to a conservative substitution at codon 122, and secondly to a point mutation in nucleotide 442 resulting in a non-conservative change of aa 148 from Arg to Leu. It would be of interest to determine whether this latter polymorphic RNA is translated and to search for a change in the functional activity of the polymorphic gp130 protein.

Finally, we investigated a cytoplasmic region of the gp130 that was shown to be sufficient to induce an IL-6-mediated signal. More recently, the Pro-X-Pro motif contained in this region was shown to be essential for association with a cytoplasmic tyrosine kinase [32]. We found point mutations in the gp130 cytoplasmic region in 2 out of 28 tumor samples. In the U266 cell line, mutation of the intracytoplasmic aa 710 converted Ser to Leu, and only the mutated allele was expressed. This sequence is different from the sequence initially reported

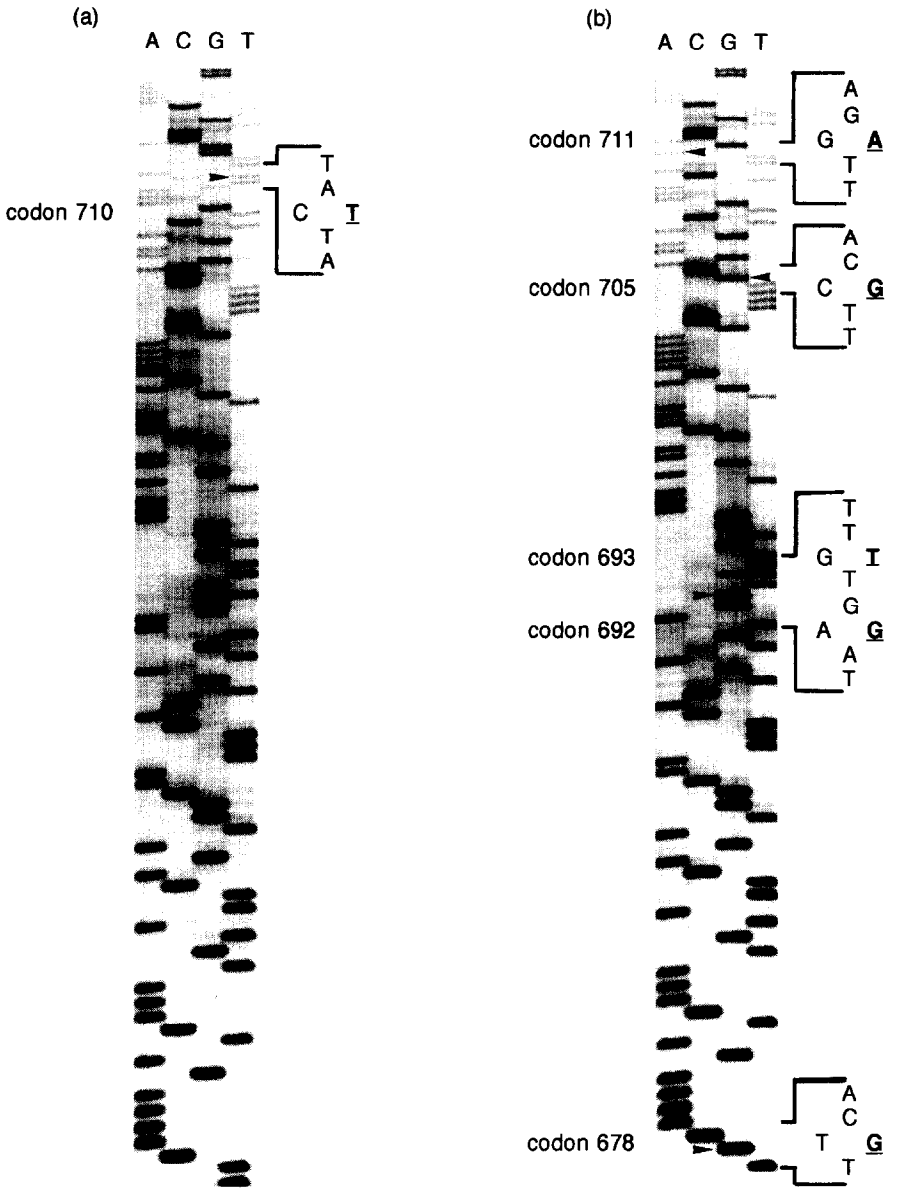

Fig. 4. Sequence analysis of gp130 cytoplasmic band shifts. Sequences are shown 5′ (bottom) to 3′ (top). The codon at which a mutation occurs is indicated. The nucleotide sites of mutation are shown by underlined letters. Arrowheads point to bands corresponding to mutated bases. (a) U266 band shift sequence; (b) patient band shift sequence.

for U266 [14]. The cell line analyzed was effectively U266, since cells were producing IgEλ. It would be interesting to investigate whether this mutation has confered a selective growth advantage. In the freshly explanted tumor sample, 5 mutations were found, one conservative of the intracytoplasmic aa 711 and 4 non-conservative of the intracytoplasmic aa 678, 692, 693 and 705. In this case, both normal and mutated alleles were expressed. The presence of 40% non-tumor cells in the tumor sample might explain why both normal and mutated alleles were expressed. Again, it would be of interest to investigate whether these mutations result in a change in gp130 functional activity.

This study analyzed the gp130 CRH and cytoplasmic domains that have been identified to date or are assumed to be essential for signal activation and transduction. As in the case of IL-6Rα, the unidentified promoter and regulating regions of the gp130 gene could be major targets of the oncogenic process.

References

[1] Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K., Asaoku, H., Tang, B., Tanabe, O., Tanaka, H., Kuramoto, A. and Kishimoto, T. (1988) Nature 332, 83–85.

[2] Klein, B., Zhang, X-G., Jourdan, M., Content, J., Houssiau, F., Aarden, L., Piechaczyk, M. and Bataille, R. (1989) Blood 73, 517–526.

[3] Klein, B., Wijdenes, J., Zhang, X-G., Jourdan, M., Boiron, J-M., Brochier, J., Liautard, J., Merlin, M., Clement, C., Morel-Fournier, B., Lu, Z-Y., Mannoni, P., Sany, J. and Bataille, R. (1991) Blood 78, 1198–1204.
[4] Levy, Y., Tsapis, A. and Brouet, J.C. (1991) J. Clin. Invest. 88, 696–699.
[5] Schwab, G., Siegall, C.B., Aarden, L.A., Neckers, L.M. and Nordan, R.P. (1991) Blood 77, 587–593.
[6] Nilsson, K., Jernberg, H. and Petterson, M. (1990) Curr. Topics Microbiol. Immunol. 166, 3–12.
[7] Caligaris-cappio, F., Bergui, L., Gregoretti, M.G., Gaidano, G., Gaboli, M., Schena, M., Zallone, A.Z. and Marchisio, P.C. (1991) Blood 77, 2688–2693.
[8] Hermann, F., Andreeff, M., Gruss, H.J., Brach, M.A., Lübbert, M. and Mertelsmann, R. (1991) Blood 78, 2070–2074.
[9] Portier, M., Rajzbaum, G., Zhang, X-G., Attal, M., Rusalen, C., Wijdenes, J., Mannoni, P., Maraninchi, D., Piechaczyk, M., Bataille, R. and Klein, B. (1991) Eur. J. Immunol. 21, 1759–1762.
[10] Kaisho, T., Oritani, K., Ishikawa, J., Tanabe, M., Muraoka, O., Ochi, T. and Hirano, T. (1992) J. Immunol. 149, 4088–4095.
[11] Bataille, R., Jourdan, M., Zhang, X-G. and Klein, B. (1989) J. Clin. Invest. 84, 2008–2011.
[12] Hirano, T., Akira, S., Taga, T. and Kishimoto, T. (1990) Immunol. Today 11, 443–449.
[13] Yamasaki, K., Taga, T., Hirata, H., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T. and Kishimoto, T. (1988) Science 241, 825–828.
[14] Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. and Kishimoto, T. (1990) Cell 63, 1149–1157.
[15] Bazan, J.F. (1990) Proc. Natl. Acad. Sci. USA 87, 6934–6938.
[16] Miyzaki, T., Maruyama, M., Yamada, G., Hatakemaya, M. and Taniguchi, T. (1991) EMBO J. 10, 3191–3197.
[17] Imler, J.L., Miyajima, A. and Zurawski, G. (1992) EMBO J. 11, 2047–2053.
[18] Yawata, H., Yasukawa, K., Natsuka, S., Murakami, M., Yamasaki, K., Hibi, M., Taga, T. and Kishimoto, T. (1993) EMBO J. 12, 1705–1712.
[19] Yoshimura, A., Longmore, G. and Lodish, H.F. (1990) Nature 348, 647–649.
[20] Yoshimura, A., Zimmers, T., Neumann, D., Longmore, G., Yoshimura, Y. and Lodish, H.F. (1992) J. Biol. Chem. 267, 11619–11625.
[21] Bass, S.H., Mulkerrin, M.G. and Wells, J.A. (1991) Proc. Natl. Acad. Sci. USA 88, 4498–4502.
[22] Longmore, G.D. and Lodish, H.F. (1991) Cell 67, 1089–1102.
[23] Fukunaga, R., Ishizaka-Ikeda, E., Pan, C-I., Seto, Y. and Nagata, S. (1991) EMBO J. 10, 2855–2865.
[24] Murakami, M., Narazaki, M., Hibi, M., Yawata, H., Yasukawa, K., Hamaguchi, M., Taga, T. and Kishimoto, T. (1991) Proc. Natl. Acad. Sci. USA 88, 11349–11353.
[25] Miura, O., Cleveland, J.L. and Ihle, J.N. (1993) Mol. Cell. Biol. 13, 1788–1795.
[26] Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. and Kishimoto, T. (1989) Cell 58, 573–581.
[27] Klein, B., Zhang, X-G., Jourdan, M., Boiron, J-M., Portier, M., Lu, Z-Y., Widjenes, J., Brochier, J. and Bataille, R. (1990) Eur. Cytokine Net. 1, 193–201.
[28] Portier, M., Molès, J-P., Mazars, G-R., Jeanteur, P., Bataille, R., Klein, B. and Theillet, C. (1992) Oncogene 7, 2539–2543.
[29] Mazars, G-R., Portier, M., Zhang, X-G., Jourdan, M., Bataille, R., Theillet, C. and Klein, B. (1992) Oncogene 7, 1015–1018.
[30] Neri, A., Murphy, J-P., Cro, L., Ferrero, D., Tarrela, C., Baldini, L. and Dalla-Favera, R. (1989) J. Exp. Med. 170, 1715–1725.
[31] Sugita, D., Totsuka, T., Saito, M., Yamasaki, K., Taga, T., Hirano, T. and Kishimoto, T. (1990) J. Exp. Med. 171, 2001–2009.
[32] Murakami, M., Hibi, M., Nakagawa, N., Nakagawa, T., Yasukawa, K., Yamanishi, K., Taga, T. and Kishimoto, T. (1993) Science 260, 1808–1810.