

## The role of cytokines, cytogenetic factors and oncogenes in the prognosis of multiple myeloma

### Rolul citokinelor, factorilor citogenetici si oncogenelor in prognosticul mielomului multiplu

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#### Abstract

*A series of cytokines, like IL-3, IL-5, IL-6, IL-10, M oncostatin, ILGF1, stimulate the growth of myelomatous cells. Some of them can worsen the prognosis of patients with multiple myeloma through their negative effects (osteolysis, inhibition of erythropoiesis) or may induce benefic effects (megakariopoiesis stimulation). The presence of chromosomal abnormalities might add to a positive prognosis (hyperdiploidia of 3, 5, 7, 9, 11, 15, 19, 21) or, conversely, could be a bad prognostic factor: t(4;14), t(14;16), del 13q and 17p13. The study of gene expression in multiple myeloma represents a modern method of identifying the genes which, through their over-expression, aggravate the patients' prognosis. Until now, approximately 33000 types of these genes have been studied in multiple myeloma.*

**Keywords:** multiple myeloma, cytokines, chromosomal abnormalities, oncogenes, prognosis.

#### Rezumat

*O serie de citokine printre care IL-3, IL-5, IL-6, IL-10, oncostatinul M, ILGF1, stimulează creșterea celulelor mielomatoase; o parte dintre acestea pot agrava prognosticul unor pacienți cu mielom multiplu prin efectele lor negative (aparitia leziunilor osteolitice, inhibarea eritropoezei) sau pot induce efecte benefice (stimularea megakariopoiezei). Prezența aberațiilor cromozomiale poate antrena un prognostic pozitiv (hiperdiploidiile perechilor 3, 5, 7, 9, 11, 15, 19, 21) sau poate constitui dimpotrivă un factor de pronostic negativ t(4;14), t(14;16), del 13q și 17p13. Studiul expresiei genelor în mielomul multiplu se constituie ca o metodă modernă prin care pot fi identificate acele gene care prin hiperexpresia lor agravează prognosticul pacienților. Au fost studiate până în prezent aproximativ 33000 de asemenea gene în mielomul multiplu.*

**Cuvinte cheie:** mielom multiplu, citokine, aberații cromozomiale, oncogene, prognostic.

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## Cytokines and multiple myeloma

Interleukin 6 (IL-6) is involved in the proliferation of the plasmablasts and in their differentiation into normal plasma cells. The adult plasma cells secrete more than 600 pg of immunoglobulins /cell/24 hours. In multiple myeloma (MM), IL-6 plays a proliferative role for the immature myelomatous cells, without being involved in their differentiation. The myelomatous cells secrete less than 10 pg of immunoglobulins/cell/24hours. Their division rate is low.

In multiple myeloma, besides the secretion of IL-6 by the myelomatous cells, an autocrine secretion by the medullary microenvironment cells appears when in close proximity of the neoplastic plasma cells. IL-6 promotes the growth of the myelomatous cells by the phosphorylation of the retinoblastoma protein (pRB). Its activation (by dephosphorylation) triggers the cease of this growth. Treatment with Dexametazone induces the apoptosis of the myelomatous cells, and beginning from here a decrease in the monoclonal proteins in more than 50% of multiple myeloma patients. Through experiments, it has been proved that this apoptosis induced by Dexametazone can be prevented by administrating IL-6.

As well as IL-6, *M oncostatin*, the leukemia inhibiting factor, through the gp130 signal transducer, acts in a similar way to IL-10 and ILGF1 (insulin like growth factor 1), by stimulating the growth of the myelomatous cells. IL-3 and IL-5 work in synergy with IL-6; the myelomatous cell secretes IL-1 $\beta$ , TNF $\beta$  and IL-6. Recently, the excessive production of IL-6 by the medullary stromal cells has been blamed on the infection of a subset of macrophages, called dendritic cells, by the Kaposhi's sarcoma associated herpesvirus (KSHV). A viral homologue of human IL-6 has been identified in the KSHV genome. Viral IL-6 is capable of stimulating the growth of myelomatous cells. These studies attempt to prove a unique pathological pattern, in

which a virus is potentially involved in neoplastic growth by infecting a non-malignant clone (1).

Besides the neoplastic proliferation, IL-6 is responsible for the osteolytic lesions in multiple myeloma, as the result of the osteoclastic cell activation; it is also involved in the occurrence of anemia, by inhibiting red blood cell proliferation and stimulating megakariopoiesis (thrombocytopenia is rarely encountered in untreated myelomas).

## Cytogenetics of multiple myeloma

Multiple myeloma is associated with significant chromosomal abnormalities that can occur at any stage of the disease. Although the proliferation rate of the disease is low, approximately one third of multiple myeloma patients have genomic rearrangements right from the onset.

By repeating the cytogenetic exams, chromosomal abnormalities are present in 50% of the patients; the rest have a normal karyotype that identifies the non-malignant plasma cells. Patients with hyperdiploidy have an average of 54 chromosomes with non-randomized additions of the 3, 5, 7, 9, 11, 15, 19, 21 chromosome pairs. Patients with hyperdiploidy have a longer life expectancy than those with hypodiploidy.

The occurrence of chromosomal abnormalities such as t(4;14), t(4;16), del 13q and 17p13 suggests a poor prognosis and imposes a high-dose chemotherapy (2). Translocation t(11;14) has a better prognosis for survival. The significance of del 13q is still unknown, as it is encountered also in patients with monoclonal gammopathy of unknown significance (MGUS) that will evolve towards multiple myeloma (3). The deletions of the chromosome arm 1p as well as the addition of arm 1q are associated with poor prognosis (4).

Recent studies have emphasized the beneficial therapeutic effects of Bortezomid in t(4;14) and del 13q (5,6).

Genetic abnormalities of the interphase cells have been identified using the FISH method. The next abnormalities are targets of molecular therapy drugs: 17p (p53), t(11;14) (Ig H, cyclin D1), t(4;14) (Ig H, FGFR3), 13q14(Rb1) (7).

The addition of the chromosome arm 1q and/or the deletion of arm 1p have been identified as mutations associated with poor prognosis; also, del 17p, t(4;14), t(14;16) and 13q34 have been identified through the FISH method as being followed by a reduced survival rate (8).

### **The role of oncogenes in the prognosis of multiple myeloma**

The malfunction of the oncogenes and the suppressor genes, which control cell proliferation and apoptosis, contributes to the pathogenic mechanism of multiple myeloma, a multi-step process that associates the alteration of the Ras oncogene, bcl2, myc and pRB proteins.

#### ***The retinoblastoma protein (pRB)***

In myelomatous cells, pRB is phosphorylated, a process that is amplified by Il-6. Cell growth signals induce the accumulation of cyclins active in G1 phase of the cell cycle, regulating the catalytic activity of cyclin-dependent kinases (CDK). The cell cycle is controlled by the sequential action of protein kinases with a similar structure to serine and threonine. The cyclin-dependent CDK4 and CDK6 kinases are involved in controlling the progression of pRB from phase G1 to phase S of the cell cycle. CDK inhibitors (CDKI) are divided into 2 subsets: a) p21, p27 and p57, with structural resemblance, that bind to a variety of CDK-cyclin complexes; b) p15INK4B, p16INK4A, p18INK4C, that form the second class of CDK inhibitors (1). The ectopic expression of these genes suppresses cell proliferation, if the pRB gene is intact. The frequent alteration of p16 in tumoral cells suggests that this

protein kinase functions as a tumoral suppressor. Hence the idea that the inactivation of p15 and p16 genes triggers the aberrant activation of CDK4 and CDK6, deflecting the cell cycle towards an unlimited aberrant proliferation.

The p18 gene is biochemically and structurally similar to the genes p15 and p16. The p18 gene interacts with CDK4 and CDK6, inhibiting the kinase activity of CDK6. Tasaka et al (2007) (9) prove that the p18 gene can be a preferential target for chemotherapy in MM. The combined deletions of genes p18 and p15 could be more significant than the deletions of gene p16 in tumorigenesis. Genes p15 and p16 are located on chromosome 9p, being specific for the proliferation of malignant B cells. Hypermethylation leads to the inactivation of p15 gene in 67% of all MM cases, and of p16 gene in 75% of cases.

#### ***The tumor suppressor protein p53***

Although the alteration of the tumor suppressor protein p53 is the most frequent mutation in human cancers, it appears in only 10% of MM cases, especially in the end-stages of the disease. On the other hand, p53 mutations are common in myelomatous cell lines obtained in vitro (80%). p53 is actively involved in DNA replication and DNA repair. Because the cytogenetics in MM is highly complex, the incidence of p53 mutations in this disease is surprisingly low. The functions of this protein could be altered by the overexpression of mdm-2 (through the PAX gene), the gene that codifies for the B-Cell-Specific Activator Protein (BSAP), required for the expression of CD19, a marker weakly expressed in myelomatous cells (10, 11).

#### ***The BCL family***

Protein-oncogenes bcl2 and bclx1 prevent the apoptosis induced by a variety of factors (glucocorticoid treatment, alkylating agent therapy, radiotherapy). Yet, they do not lower the rate of cell proliferation. The BCL family also contains proapoptotic factors, such as bax, bclx2 and bad. The active status of the

cell is defined by the ratio between antiapoptotic and proapoptotic factors.

In MM the frequency of t(14;18) is of only 2%, although in myelomatous cell lines there are high levels of this oncogene; bcl2 is a regulating factor for myelomatous cell survival in the early stages of the disease, in which there is a low rate of cell proliferation. The effect of bcl2 probably decreases in advanced stages, when the proliferation rate for the malignant cells increases. The levels of this oncogene are well correlated with the response efficiency to interferon alpha.

#### ***p21Ras protein***

This protein is a signal transducer that limits cell growth and proliferation. p21 binds GTP, depending on its concentration in the tissue. IL-6 is a powerful growth factor, with an antiapoptotic effect on the myelomatous cells, using gp130 as a signal transducer. This signal transducer determines the activation of the Janus kinase (JAK), as well as the activation of the Ras gene kinase. The punctiform mutations of the Ras gene are more frequent in codons 12, 13 and 61, playing a role in the constitutive activation of this gene, therefore giving independence to the myelomatous cells from the action of exogenous IL-6.

#### ***c-myc gene***

It is a gene associated with the progression of the cell cycle. In the circumstances where the growth stimulating factors are missing, the expression of this gene is closely related to apoptosis. Malfunctions of this gene have been observed in most patients with MM, with present aberrations in the translation and transcription mechanisms. A large part of these MM patients have an aberrant messenger-ARN at the level of this gene, due to the intervention of an unusual promoter (P0), and never of the common promoters P2 and P1. High concentrations of c-myc in this disease could be explained by the alteration of the transcriptional mechanisms at the level of cyclin 1.

### **Gene expression profile in patients with multiple myeloma (GEP)**

Although it is still an experimental method, GEP has already been carried out in approximately 6800 genes obtained from myelomatous cells of recently diagnosed patients. Genes of some patients with MGUS were also analyzed. The genetic profile that suggests a poor prognosis is accompanied by the presence of cytogenetic abnormalities and the rising of  $\beta$ 2-microglobuline levels. Although in MGUS there are no myelomatous cells present in the hematogenous bone marrow, only plasma cells with the ability to transform themselves, this peculiarity gives MGUS the character of an "open malignant disease". The transformation of MGUS into multiple myeloma is always accompanied by disturbances of the medullary microenvironment, as well as of the immune regulation mechanisms that initiate at cellular level the genetic aberrations which allow the transformation of the normal plasma cell into a myelomatous malignant cell.

GEP tests on B CD19+ lymphocytes and CD138+plasmocytes of medullary origin reveal the fact that both cells possess the "foot-print" of a malignant disease. It has been demonstrated that only 40% of the MM patients have at least one of the 5 translocations which characterize the immunoglobuline heavy chain locus. Therefore, the presence of the extremely frequent deletion 13 is correlated with a short term survival.

GEP tests on stage I myelomatous patients and on the ones in stage III showed a high expression of the Wnt signal inhibitor of DKK1 and an increased medullar infiltration. Using GEP could yield information on the average disease-free survival period in MM patients.

A trial aimed to randomize the patients treated with Thalidomide versus the ones not treated with this drug was performed in the USA (NCI) between 1998 and 2003. A prognosis risk model was developed with the help

of the signature expression revealed by GEP.4 prognosis subgroups characterized by differences in chromosomal aberrations were identified, out of which hypodiploidy, trisomy 11 and t(14q;32) are of significance. These chromosomal aberrations correlate with other paraclinical indicators such as: serum albumin <35g/L,  $\beta$ 2-microglobuline > 4mg/L, serum creatinine > 2mg/dL, the presence of osteolytic lesions. These indicators are directly correlated with the mean disease-free survival period.

The majority of the patients with genetic profile: CCND1 is associated with a normal karyotype. CCND1 expression, the presence of trisomy 11 or hyperdiploidy or normal karyotypes are prognosis subgroups with low relative risk of rebound. A high expression of MMSET / MAF-B defines poor prognosis groups. CCND1 expression frequently associates with normal karyotypes. The use of the COX model in the multivariable analysis of the prognosis factors identified through GEP in MM proved that only around 100 genes, from the 10000 studied, are associated with an extended mean survival period.

The study of deletion 13 through GEP led to identifying 3 significant genetic profiles: the CHC1-L gene, the RAN gene and the ZHX2 gene. A high level of RAN gene is accompanied by a high rebound risk, meanwhile a high expression of the CHC1-L gene lowers the rebound risk (12, 13). The presence of deletion 13, revealed through the FISH method, is a poor prognosis factor.

The overexpression of the RAN gene accompanied by the weak expression of the ZHX2 and CHC1-L genes form a genetic combination of unfavorable prognosis. The CHC1-L gene is localized at the 13q14 level, in a region which functions as the tumoral suppressor protein of the myelomatous clone. A decrease in the concentration of this gene is a major argument in stating the aggressive character of the disease, as this gene is actually a tumor suppressing gene. By cloning the ZH2 gene, its in-

hibitive function of NFY transcription was proven; the gene is located in the proximity of the myc oncogene.

Multiple myelomas accompanied by aberrations of the 8 chromosomal pair are linked to the high risk of rebound and death, even with high-dose cytostatic treatments and peripheral stem cells transplant. The weak expression of the ZHX2 gene provides a very favorable prognosis.

Nowadays, GEP studies that evaluate a higher number of genes (approximately 33000) in order to classify MM by molecular profile (14) are underway.

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