

Review

Cell biological roles of IL-10 in myeloma cells

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IL-10, along with interleukin (IL)-6, are considered two of the most important cytokines that regulate the proliferation and cellular characteristics of myeloma cells. It remains unclear from the clinical data how levels of IL-10 in serum at various stages of myeloma are related to clinical manifestations of this disease. Several studies have reported that IL-10 affects myeloma cells by stimulating secondary signals for cell proliferation through oncostatin M (OSM) and IL-11. In experiments using human myeloma cell lines that were established in our laboratory, IL-10 seemed to be expressed in half of myelomas simultaneously with OSM, and was correlated with c-maf, a transcription factor that has been known to be overexpressed in myelomas with t (14 ; 16) (q32 ; q23). In addition, IL-10 abolishes all trans-retinoic acid (ATRA)-induced growth inhibition of myeloma cells. The expression and production of IL-10 in myeloma patients may be important for sub-categorization and to establish case-oriented therapies.

Key words myeloma, interleukin 10, c-maf, ATRA, gene expression

INTRODUCTION

The proliferation of malignant human myeloma cells is believed to be supported by a cytokine network that surrounds the cells¹⁻⁵. Interleukin (IL)-6 has been reported to contribute to myeloma cell survival and proliferation via an autocrine and/or paracrine mechanisms⁶⁻¹⁰. In addition, the importance of IL-10 and its receptor, as well as receptors for gp130-related cytokines, as factors for myeloma cell growth arose from clinical and experimental investigations. The role of IL-10 may be especially critical in myeloma cells that escape from an IL-6-dependent proliferation loop through malignant progression. In this article, we discuss the clinical importance of IL-10 in myeloma and introduce our current findings related to the biological roles of IL-10 in myeloma cells.

1. *Cytokines that induce proliferation of myeloma cells*

Since initial reports identifying IL-6 as one of the main growth factors for myeloma cells⁶, several studies have demonstrated a relationship between serum IL-6 and disease activity¹¹⁻¹³. IL-6 induces the proliferation of myeloma cells via a paracrine pathway rather than an autocrine loop and is secreted and produced from surrounding stromal cells, as well as from the myeloma cells themselves⁶⁻¹⁰. There have been several reports that expression of CD126 (IL-6 receptor (R)- α chain) is limited in malignant plasma cells but not in normal cells¹⁴. In addition, there have been reports of several proliferation factors for myeloma cells such as insulin-like growth factors (IGF)¹⁵⁻¹⁷, IL-15¹⁸, and IL-10¹⁹. There is no doubt that the proliferation of myeloma cells is supported by an array of cytokines and by a network between myeloma cells and stromal cells surrounding these malignant cells.

2. *IL-10 as a proliferation factor for myeloma cells*

Klein, *et al.* have made several attempts to explore the role of IL-10 in myeloma cell biology^{19,20-23}. They initially reported that IL-10

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stimulated two of four cytokine (IL-6)-dependent cell lines that proliferated in the absence of IL-6 to obtain IL-10 dependent cell lines, although IL-10 did not induce any differentiation or increase in immunoglobulin synthesis in these four cell lines¹⁹. In addition, they reported that IL-10 induced expression of the IL-11 receptor, making it sensitive to IL-11²⁰, and stimulated oncostatin M (OSM), another gp130-related cytokine, via an autocrine loop in myeloma cells²¹. Based on these results, they suggested that the action of IL-10 to promote growth in myeloma cells is mediated through OSM²².

3. Expression and production of IL-10 in myeloma cells

Because IL-10 is an 18 kD glycoprotein produced by activated T cells, monocytes, B cells, and thymocytes²³, it should be possible to identify the type of cell that elevates serum IL-10 in myeloma patients. To address this issue, we examined the gene expression and production of IL-10 in various human myeloma cell lines established at our laboratory²⁴⁻²⁷. Half of the 10 myeloma cell lines studied, including the internationally well-characterized U266 and RPMI8226 lines, expressed detectable levels of the IL-10 transcript. All of the IL-10-expressing lines examined (n=7) produced and secreted IL-10 into culture medium²⁸. In addition, we examined IL-10 gene expression in 15 primary specimens derived from the bone marrow of myeloma patients at initial diagnosis and from 5 normal bone marrow specimens. The IL-10 message was detected in all specimens, and 4 out of 15 myeloma specimens showed a higher level of expression. These findings suggested that IL-10 was expressed and produced in one-third to one-half of myeloma cells, and also in stromal cells.

We also examined the message levels of gp130-related cytokines such as OSM, IL-11, leukemia inhibitory factor (LIF), and their receptors. As Klein, et al. suggested, all cells that expressed OSM were IL-10-expressing lines, whereas the IL-11 message was detected in all lines studied. In addition, the IL-6 message was observed in both IL-10 expressers and non-expressers. In contrast, some IL-10 expressers were not IL-6 expressers. These results supported the notion that IL-10 enhanced OSM-induced myeloma cell proliferation.

4. Relationship between expression of cytokines and myeloma-related genes overexpressed due to chromosomal translocations

As shown in Fig. 1, we examined the relative gene expression levels of cytokines and their receptors and myeloma-related genes overexpressed due to chromosomal translocations, using semi quantitative multiplex RT-PCR (SQ-MP-RT-PCR) among myeloma cell lines established at our laboratory.

IL-10 was expressed strongly in the cell lines KMM-1, KMS-11, KMS-21PE, KMS-24 and KMS-28PE, and weakly in KMS-21BM, KMS-26, KMS-27, and KMS-34. IL-10R was detected in all lines at various degrees. Although IL-6R and gp130 were expressed ubiquitously in all lines, the IL-6 message was detected in only half of these lines. Cyclin D1 was highly expressed in KMS-12PE, KMS-12BM, KMS-21PE, KMS-21BM, and KMS-27. Most of these cell lines were confirmed to have t (11 ; 14) by the fluorescent in situ hybridization (FISH) method^{26,35}.

As we reported previously, cyclin D2 expression showed an inverse correlation with cyclin D1 expression³⁶. Most cyclin D1 expressers did not have the cyclin D2 message. In addition, cyclin D3 expression was detected only in KMM-1, in which t (6 ; 22) (p21 ; q11) was analyzed recently and KMS-18, under the SQ-MP-RT-PCR conditions. FGFR3 was overexpressed in KMS-11, KMS-18 (both lines were confirmed to have this translocation by FISH^{28,34}), KMS-28PE, KMS-28BM, and KMS-34. In addition, c-maf was overexpressed in KMM-1, KMS-11 (in which t (14 ; 16) (q32 ; q23) was initially reported³³), KMS-26, and KMS-34, whereas the gene was faintly expressed in most of the lines studied, except KMS-18, under the same conditions. This may have been because c-maf was a transcription factor³⁷ and might its expression might be required for cell proliferation in tumor cells. However, MUM1 was ubiquitously expressed in the myeloma lines studied, because none of the cell lines used had been confirmed to possess t (6 ; 14) (p25 ; q32).

To determine the relationships among these genes, we analyzed the significance of relative expression levels (the intensity of RT-PCR product for the target gene divided by that of the β -actin gene) using Spearman's rank correlation test.

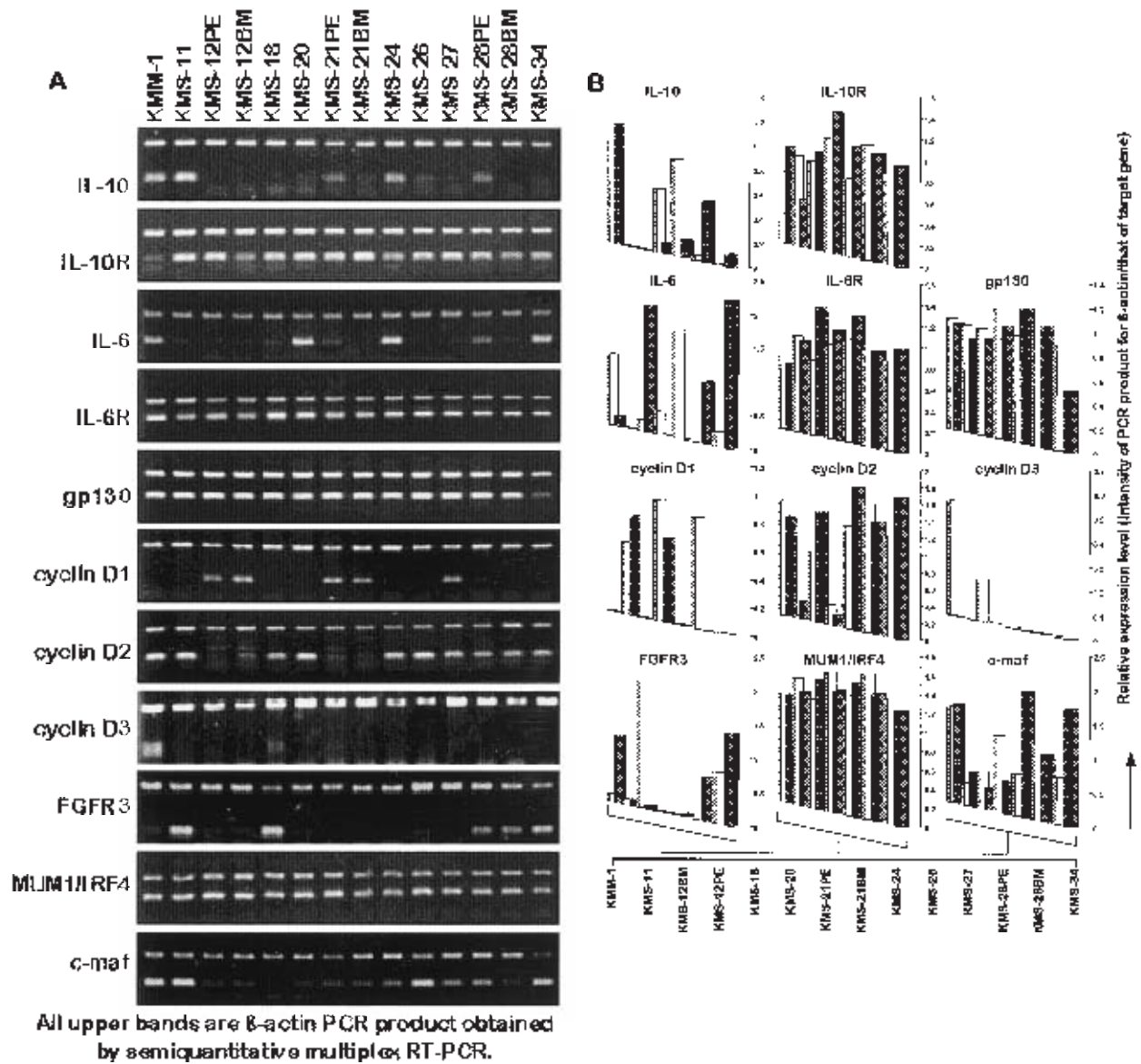


Fig. 1. [A] Gel images of semi-quantitative multiplex RT-PCR for cytokines (IL-10 and IL-6), their receptors (IL-10R, IL-6R, and gp130), and myeloma-related genes overexpressed due to chromosomal translocations (cyclin D1, cyclin D2, cyclin D3, FGFR3, c-maf, and MUM1/IRF4). RNA extraction, cDNA synthesis, and multiplex reverse transcription-polymerase chain reaction (MP-RT-PCR) were performed as described previously. MP-RT-PCR was used to examine the mRNA levels of cell cycle regulator genes in the cell lines. β -actin, the housekeeping control gene, and the target gene were amplified in a single reaction. The ratios of β -actin, target gene primer-sets and the number of PCR cycles were determined to amplify both products logarithmically and in relatively similar amounts. The procedure followed for MP-RT-PCR also has been reported previously. After visualization of MP-RT-PCR products electrophoresed on a 1.2% agarose gel stained with ethidium bromide, gel images were obtained using the FAS-II UV-image analyzer (TOYOBO Co. Ltd., Tokyo, Japan), and the densities of the products were quantified using Quantity Onetm version 2.5 (PDI Inc., Huntington Station, NY). [B] The relative expression level of each target gene in individual lines was calculated as the density of the product of that gene divided by β -actin gene activity derived from the same MP-RT-PCR.

There were several significant inverse correlations among translocation-related genes such as between cyclin D1 and FGFR3 ($\sigma = -0.578$, $p = .0373$), which mean that t (11; 14) and t (4; 14) did not overlap in the lines studied, and between cyclin D1 and cyclin D2 ($\sigma = -0.561$, $p = .0431$), as

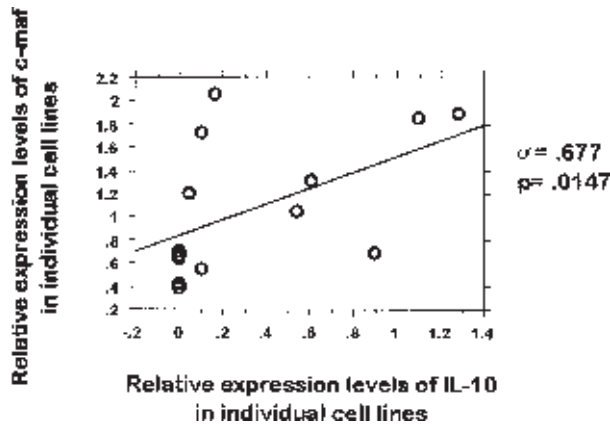


Fig. 2. The positive correlation ($\sigma = .677$, $p = .0147$) between the relative expression levels of IL-10 and c-maf in the human myeloma cell lines that were studied.

mentioned above and reported previously³⁹.

However, the relative expression level of IL-10 was positively correlated with c-maf expression, as shown in Fig. 2. Considering c-maf as a transcription factor, the expression and production of IL-10 inducing myeloma cell proliferation may be caused by an over-expression of c-maf. Further study is required to confirm this hypothesis.

5. The role of IL-10 in all trans-retinoic acid (ATRA) treatment of myeloma cells

Recently, it was disclosed that ATRA inhibits myeloma cell growth by down regulating the IL-6/IL-6R auto/paracrine loop^{38,39}, and up regulating the p21/Cip1 cyclin-dependent kinase-inhibitor (CDK-I)^{40,41}, inducing apoptosis and decreasing expression of Bcl-2, an anti-apoptotic protein⁴². In addition, several clinical trials of ATRA, with or without biological modifiers such as interferon α or dexamethasone, have been reported^{43,44}.

We recently reported that 2 out of 10

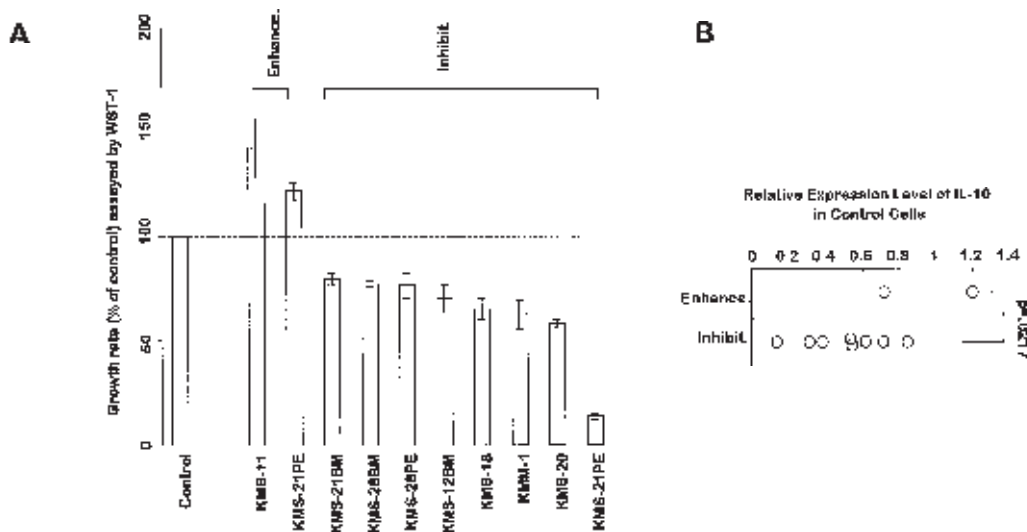


Fig. 3. [A] The WST-1 assay was used to characterize the growth of myeloma cells cultured with 10^{-7} M ATRA. A Premix WST-1 Cell Proliferation Assay System (Takara Biochem., Tokyo, Japan) was used. Water-soluble tetrazolium salt, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, a monosodium salt (WST-1), was added to the culture for the final four hours. Then, the absorbance ($A_{450nm} - A_{600nm}$) of Formosan, the product of the reduction of WST-1 by mitochondrial dehydrogenase, was measured by an ELISA reader and cell growth was determined as a percentage of control. [B] The relative expression levels in the control cultures (cells cultured without ATRA) were compared between growth enhanced lines (KMS-11 and KMS-21-PE) and inhibited lines. The enhanced lines had significantly higher expression of IL-10.

myeloma cell lines were stimulated to grow by 10^{-7} M ATRA, *in vitro*⁴⁵, as shown in Fig. 3A. Thus, we sought factors related to the growth enhancement in myeloma cells. IL-10, whose message was abundant in control cells (no ATRA treatment) emerged as a candidate. As shown in Fig. 3B, two lines whose growth was enhanced by ATRA showed significantly higher relative expression of IL-10 than lines in which growth was inhibited. To explore the possibility that

IL-10 abolishes the growth inhibition effects of ATRA on myeloma cells, we observed the growth characteristics of KMS-21PE cells that were stimulated by ATRA and the IL-10 expresser, cultured with or without ATRA and 200 μ g/ml of anti-IL-10 monoclonal antibody (MoAb). As shown in Fig. 4, anti-IL-10 MoAb suppressed growth and this suppression was overcome by ATRA-induced growth enhancement. In contrast, recombinant IL-10 recovered the growth inhibition induced by ATRA at low concentrations (10^{-9} and 10^{-8} M), except at 10^{-7} M in KMS-20 cells, which were inhibited by ATRA, as shown in Fig. 3A, and did not express IL-10 (Fig. 5). These findings indicated that IL-10 overcame the ATRA-induced growth inhibition in myeloma cells and that cells that produced a large amount of IL-10 were affected by the growth suppression effects of ATRA.

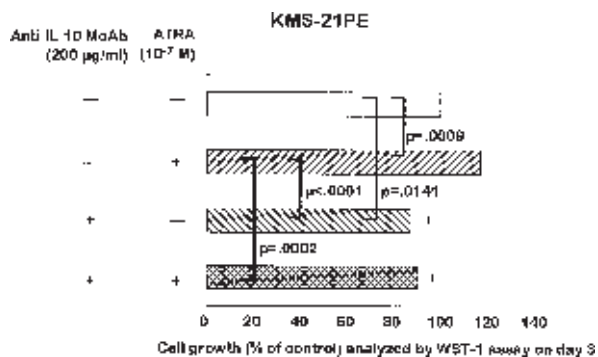


Fig. 4. KMS-21-PE cells, growth enhanced by ATRA, were cultured with 0 and 10^{-7} M ATRA, with or without supplementation by 20 μ g/ml of anti-IL-10 MoAb, for two days. The WST-1 assay was performed to examine growth properties.

6. Serum IL-10 levels in myeloma patients

An initial report published in 1992 demonstrated that IL-10 was detected in 20% of patients with early stage multiple myeloma (MM), but in only 2.5% of those with advanced disease⁴⁶. In addition, IL-10 was not detected in the serum of individuals with monoclonal gammopathy with

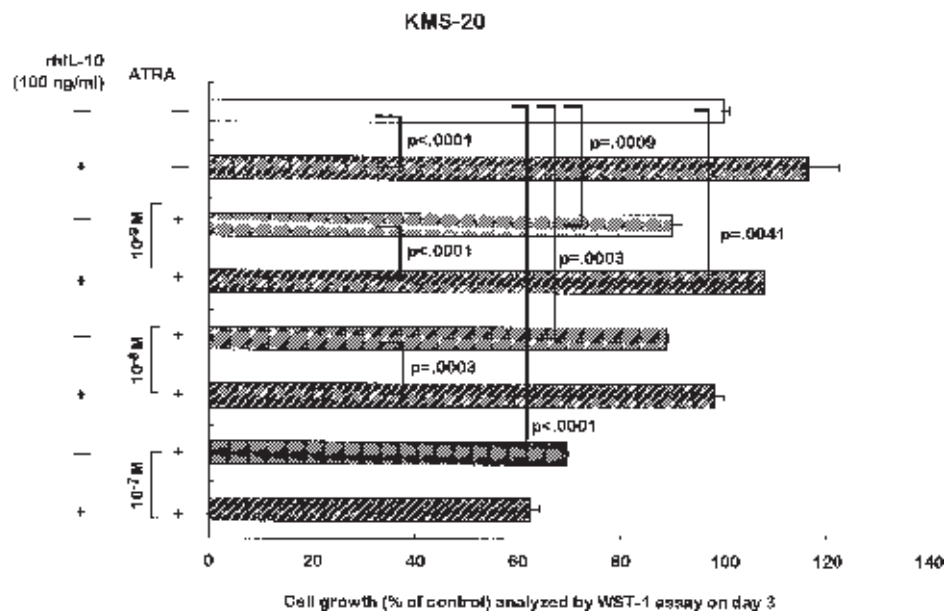


Fig. 5. Anti-IL-10 MoAb suppressed the growth of KMS-21-PE cells. This suppression was overcome by ATRA-induced growth enhancement. In contrast, recombinant IL-10 recovered the growth inhibition induced by ATRA at low concentrations (10^{-9} and 10^{-8} M), but not at 10^{-7} M, in KMS-20 cells.

undetermined significance (MGUS). This suggested that IL-10 and IL-6 are involved in human MM, though the serum level did not correlate with disease activity or progression. Furthermore, it was mentioned that serum IL-10 might be associated with a good prognosis.

Another study examined serum IL-10 concentrations in plasma cell dyscrasia and found detectable levels of serum IL-10 in 20.9%, 9.9%, and 7.5% of myeloma, MGUS, and normal subjects, respectively⁴⁷. However, a detailed analysis revealed that the IL-10 concentrations did not differ significantly between controls and patients with plasma cell dyscrasia, between patients with MGUS and those with MM, between early vs. advanced MM, nor between patients in different phases of the disease.

In contrast to these studies, a recent report showed significantly elevated serum IL-10 levels in myeloma patients compared with healthy individuals⁴⁸. This report also demonstrated that the levels of IL-10 correlated positively with an advanced stage of disease estimated according to the Salmon and Durie classification. Higher levels of IL-10 were found in patients with light chain disease and hypercalcemia, and correlated with elevated concentrations of C-reactive protein (CRP). This study also showed a positive correlation between IL-10 and IL-6 serum levels in MM patients. Despite these investigations it is still not clear what types of cells (myeloma or stromal) produce IL-10, and what role IL-10 plays in myeloma cell biology.

Conclusion

IL-10 is an important cytokine to understand myeloma cell biology. It acts as a proliferation factor through up-regulation of the OSM loop. Its expression may be affected by c-maf (a transcription factor and a product of a myeloma-related gene overexpressed in a disease-specific chromosomal translocation). In addition, IL-10 abolishes ATRA-induced myeloma cell growth inhibition. Studies of the expression and production of IL-10 in myeloma patients may be important for sub-categorization and the establishment of a case-oriented therapy for these patients, since such information would prove helpful in characterizing myelomagenesis and the progression of myeloma.

These observations and a summary of the literature are shown schematically in Fig. 6. Although the figure is complicated, its complex-

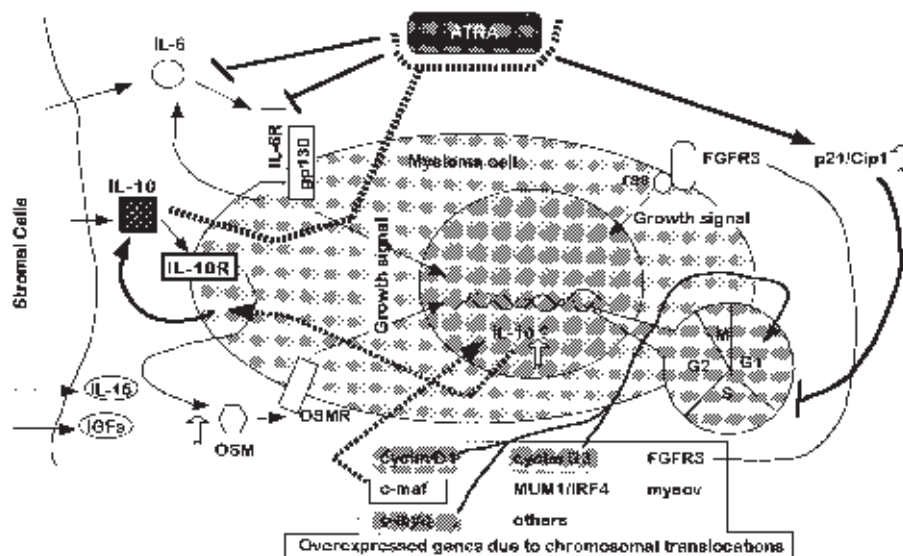


Fig. 6. The cell biological roles of IL-10 in myeloma cells are shown schematically. IL-10 produced from myeloma and stromal cells induces OSM up-regulation and subsequently induces growth of myeloma cells. Over-expression of various genes caused by chromosomal translocations affects cell cycle progression (cyclin D1, cyclin D3, and c-myc) and the ras-related growth signal (FGFR3), and may influence IL-10 production (c-maf). IL-10 abolishes ATRA-induced growth inhibition in myeloma cells.

ity probably arises from the scarce information definitively elucidated from myeloma cell biology. In addition to the factors shown in the figure, various alterations in molecules, such as angiogenic factors (including vascular endothelial cell growth factors (VEGF) and FGFs⁴⁹⁻⁵¹), the silencing of CDK-Is (such as p16/ink4a due to hypermethylation^{52,53}), and mutation of Fas receptor to protect myeloma cells from immunological attacks caused by cytotoxic T cells and NK cells⁵⁴, have been considered as important in myelomagenesis and the progression of myeloma cells. Clarification of the relationships among these various factors, including overexpressed genes caused by chromosomal translocations, may lead to the discovery of molecular targets for novel therapeutic tools against myeloma.

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