Paradoxical Growth Enhancement of Myeloma Cells Induced by Interferon-α

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ABSTRACT. To evaluate and generalize the effects of IFN-α on myeloma cells, their growth features were studied using 14 myeloma cell lines established at Kawasaki Medical School. Although 5 out of 14 lines showed significant growth inhibition, two lines revealed growth enhancement. Furthermore, this growth enhancement disappeared when JAK/STAT signaling was inhibited using AG490. Further investigations of which molecules are involved in this growth enhancement and what cellular characteristics affect the growth response in myeloma cells against IFN-α are necessary.

Key words : myeloma — IFN-α — JAK/STAT

Multiple myeloma remains an incurable hematological malignancy.[1-4] Since the introduction of melphalan and prednisolone (MP therapy) to improve the clinical response to myeloma, large numbers of multi-drug chemotherapies have been reported.[5,6] However, there has been little improvement in outcome during the past three decades. Recently, interferon-α (IFN-α) has been used as a chemotherapeutic agent for myeloma with or without other cytoreductive drugs.[7-13] There have been reports of its clinical effectiveness, particularly as a main agent for maintenance therapy.[7,8] Several investigations including our own have shown the cellular biological effects of IFN-α on myeloma cells to be induction of apoptosis,[14-16] down-regulation of interleukin (IL)-6 receptor (R),[17-20] and an inhibition of the IL-6 dependent signaling pathway.[17-20]

In this study, we examined the growth features of human myeloma cell lines established in our laboratory cultured with IFN-α to generalize its effects. Interestingly, a few lines showed paradoxical growth enhancement transducing via the JAK/STAT pathway. Based on these results, cases should be taken in using this agent clinically.

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MATERIALS AND METHODS

Cell lines used in this study

Fourteen human myeloma cell lines established at Kawasaki Medical School were used in this study.[21-25] Information on these lines is given in Table 1. The culture conditions have been reported previously.[22-25]

TABLE 1. Information on human myeloma cells established at Kawasaki Medical School

	Cultured tissue	Particular karyotype	Overexpressed gene	Other characteristics
KMM-1	subcutaneous tumor	t(8 ; 14)(q24 ; q32)		IL-10 production
KMS-11	pleural effusion	t(4 ; 14)(q16.3 ; q32.3)	FGFR3(with mutation)	IL-10 production
		t(14 ; 16)(q32.3 ; q23)	c-maf	
KMS-12PE	pleural effusion	t(11 ; 14)(q13 ; q32)	cyclin D1	amylase production(+)
				CD7(+)
KMS-12BM	bone marrow	t(11 ; 14)(q13 ; q32)	cyclin D1	amylase production(−)
KMS-18	peripheral blood	t(4 ; 14)(q16.3 ; q32.3)	FGFR3(with mutation)	
KMS-20	bone marrow	-13		familial case
		t(1 ; 16)(q21 ; q22)		
KMS-21PE	pleural effusion	t(11 ; 14)(q13 ; q32)	cyclin D1	CD7(+)
		t(8 ; 14)(q24 ; q32)		IL-10 production
KMS-21BM	bone marrow	t(11 ; 14)(q13 ; q32)	cyclin D1	CD7(+)
		t(8 ; 14)(q24 ; q32)		
KMS-24BM	bone marrow	t(1 ; 6)(p13 ; q23)	n.e.	IL-10 production
KMS-26	pleural effusion	now examining(n.e.).	n.e.	n.e.
KMS-27	peripheral blood	n.e.	n.e.	n.e.
KMS-28PE	pleural effusion	n.e.	n.e.	n.e.
KMS-28BM	bone marrow	n.e.	n.e.	n.e.
KMS34	pleural effusion	n.e.	n.e.	n.e.

FGFR3 ; fibroblast growth factor receptor 3

Growth assay

The effect of IFN-α on myeloma cell growth was examined using the WST-1 assay.[26] Briefly, cells were cultured with 0, 50, 100, 200 units/ml of recombinant IFN-α-2b (Intron A®: kindly provided by Schering-Plough K.K., Osaka, Japan) for two days and applied to a Premix WST-1 Cell Proliferation Assay System (Takara Biochem., Tokyo, Japan). 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 which is 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 the control (no addition of IFN-α). In addition, the effects of various inhibitors for signaling pathways ; i.e., AG490 (10 mM) ; JAK-2 inhibitor, Wortmannin (10 μM) ; PI3-K (phosphatidylinositol 3 kinase) inhibitor, and PD98059 (1 mM) ; MAP kinase (MEK1) inhibitor, on IFN-α-induced growth enhancement in the KMS-34 line were also assayed using WST-1.

Statistical analysis

The statistical significance of the changes in the growth of myeloma cells

cultured with various concentrations of IFN-α and the effects of various signaling inhibitors were analyzed using Scheffe's F test.

RESULTS

Growth of myeloma cell lines cultured with IFN-α

As shown in Fig 1, the KMS-20 line was the most sensitive to IFN-α and revealed remarkable growth inhibition even in the lowest concentration of IFN-α studied. The KMS-27, KMS-21BM, KMS-12BM, and KMS-18 lines showed significant IFN-α-induced growth reduction and this growth inhibition seemed dose-dependent.

There were no changes in the growth features of the KMS-11, KMS-21PE, KMS-28PE, and KMS-28BM lines among the studied concentrations of IFN-α (no change group : NC). In addition, although KMM-1, KMS-12PE and KMS-26 showed growth enhancement in one or two of the IFN-α concentrations studied, these lines might be placed in the NC group because enhancement in these lines was statistically significant but slight and dose-independent.

It was noteworthy that the KMS-24BM and KMS-34 lines paradoxically showed growth enhancement even in the lowest concentration of IFN-α studied and this enhancement was of a similar degree at the other concentrations studied with these lines (Fig 2).

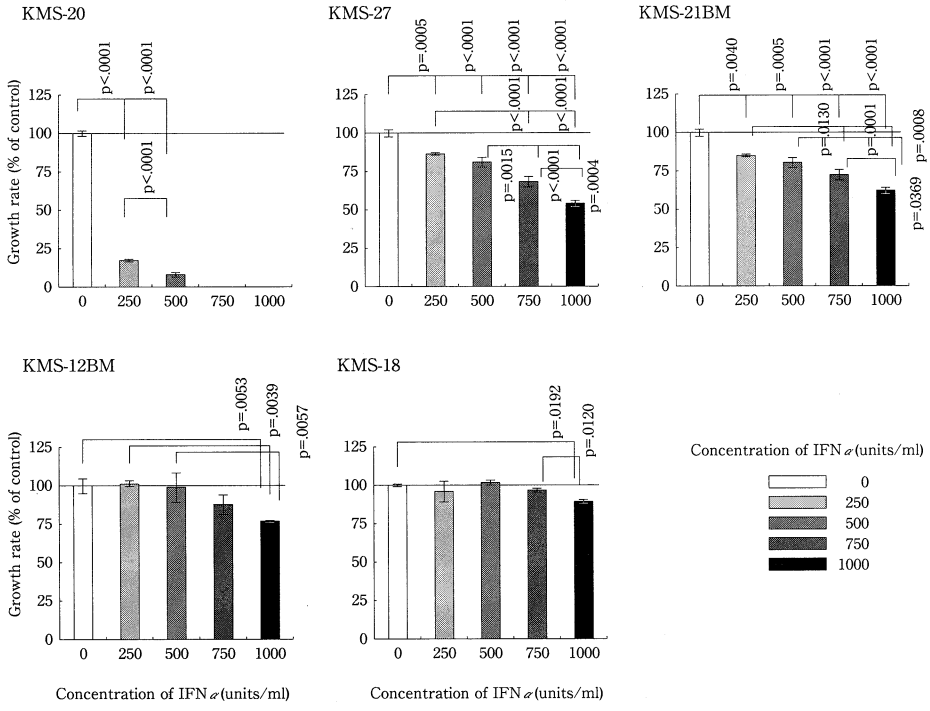

Fig 1. Five human myeloma cell lines (KMS-20, KMS-27, KMS-21BM, KMS-21BM, and KMS-18) revealed growth reduction when cells were cultured with various amounts (0, 250, 500, 750, and 1000 units (u)/ml) of IFN-α. The growth was assayed by WST-1. The growth rate was presented as the % of the control cultured without IFN-α.

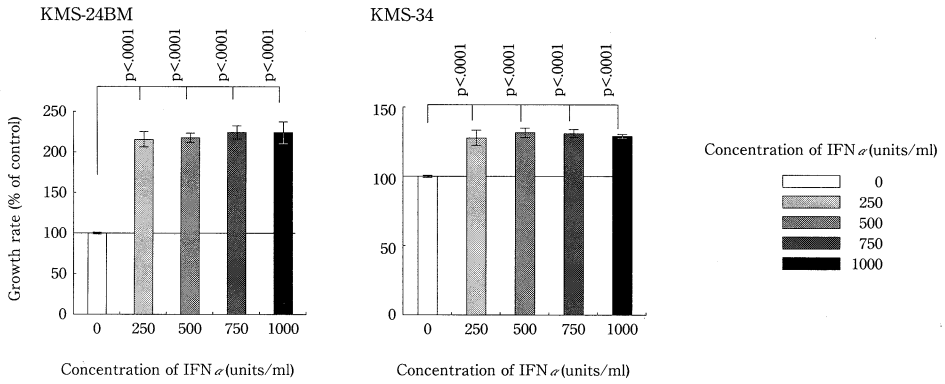

Fig 2. KMS-24BM and KMS-34 myeloma lines showed growth enhancement when cultured with various amounts of IFN-α.

Effects of signaling inhibitors in IFN-α-induced growth enhancement in KMS-34

To assess which signaling pathway was involved in IFN-α-induced growth enhancement in KMS-34 cells, the effects of various signaling inhibitors were examined. As shown in Fig 3, PD98059 (1 mM) and Wortmannin (10 μM) did not disturb IFN-α-induced growth enhancement in KMS-34 cells, but when AG490 (10 mM) was added to the culture, KMS-34 showed a loss of growth enhancement due to IFN-α.

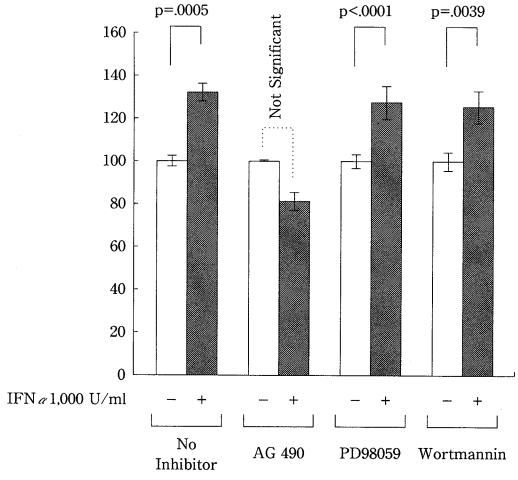

Fig 3. KMS-34 cells were cultured with or without 1000 u/ml of IFN-α. Various signaling inhibitors; i.e., AG490 (10 mM); JAK-2 inhibitor, Wortmannin (10 μM); PI3-K (phosphatidylinositol 3 kinase) inhibitor, and PD89059 (1 mM); MAP kinase (MEK1) inhibitor, were added to the culture. Y-axis shows growth rate (% of control).

DISCUSSION

One of the most important problems in myeloma therapy is a lack of

improvement in overall survival rates,[1-6] although remission induction has gotten better since the introduction of VAD therapy[27-29] and hematopoietic stem cell transplantation.[30,31] Recently, there have been several reports of new agents for myeloma therapy such as all trans retinoic acid (ATRA),[32,33] thalidomide,[34,35] bisphosphonate,[36,37] and ascetic acid.[38,39] However, these agents are still being used on a trial basis.

IFN-α has come into common clinical use during this decade, particularly for maintenance therapy after the achievement of good remission. However, no standards for usage of IFN-α have been established. It has not been decided whether or not it should be used as a single agent, combined with other cytoreductive agents, or used after stem cell transplantation. In addition, it has not been clarified how IFN-α acts on human myeloma cells.

Although there have been several reports concerning the mechanism involved in the growth inhibition of myeloma cells induced by IFN-α, little is known regarding growth enhancement of these cells. The results of this study and a few previous investigations have shown that there are myeloma cells in which growth is enhanced by IFN-α.[14,18] However, there has been little clarification of why IFN-α-induced growth enhancement in myeloma cells occurs and how the growth signal is transduced. Our results clearly demonstrated that the JAK/STAT pathway is involved in this growth enhancement, but that the MAK kinase and PI3-K pathways are not.

Even after obtaining these results, it remains unclear which genes are up- or downregulated by IFN-α, which receptors on the cell surface of myeloma cells play an important role in the response to IFN-α, and which cellular characteristics of myeloma cells make difference in reduction or enhancement of their growth by IFN-α. It would be considered that IL-6R/gp130 receptor complex possesses an important role in alteration of myeloma cell growth caused by IFN-α, because main signaling pathway from this complex is JAL/STAT one. In addition, down-regulation of IL-6R,[17-20] and an inhibition of the IL-6 dependent signaling pathway[17-20] have been reported to be involved in growth inhibition of myeloma cells caused by IFN-α. Clinically, it may be helpful to analyze the relationship between response to IFN-α and serum IL-6 or soluble IL-6R values in myeloma patients, and surface expression of IL-6R (CD126), gp130 (CD130) in primary myeloma cells. Future investigations should be focused on dysregulation of this signaling pathway to recognize primary myeloma cells which are sensitive to IFN-α therapy clinically.

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