

# Mitogenic Signals Initiated via Interleukin-6 Receptor Complexes in Cooperation with Other Transmembrane Molecules in Myelomas

Hideaki Ishikawa, Naohiro Tsuyama, Masanori Obata and Michio M. Kawano

Cytokines exert multiple biological functions through binding to their specific receptors that triggers activation of intracellular signaling cascades. The cytokine-mediated signals may produce variable and even opposing effects on different cell types, depending on cellular context that is also dictated by the differentiation stage of the cell. Multiple myeloma (MM) is a monoclonal proliferative disorder of human plasma cells. Myeloma cells appear to include mixed subpopulations in accordance with the expression of their surface antigens, such as CD45. Although interleukin-6 (IL-6) is widely accepted as the most relevant growth factor for myeloma cells, only a few subpopulations of tumor cells, such as CD45<sup>+</sup> immature cells, proliferate in response to IL-6. The activation of both signal transducer and activator of transcription (STAT) 3 and extracellular signal-regulated kinase (ERK) 1/2 is not sufficient for IL-6-induced proliferation of myeloma cells that requires the src family kinase activation associated with a rapid translocation of CD45 to lipid rafts. The CD45 expression renders myeloma cells competent for not only mitogenic but also apoptotic stimuli, resulting in either proliferation or apoptosis of CD45<sup>+</sup> myeloma cells dependently upon the circumstantial stimuli. In contrast, in CD45<sup>-</sup> myeloma cells highly expressing IL-6 receptor  $\alpha$  chain (IL-6R $\alpha$ ), IL-6R $\alpha$  and insulin-like growth factor (IGF)-I receptors exist on plasma membrane in close proximity, facilitating efficient assembly of two receptors in response to IL-6. The synergistic effects of IL-6R $\alpha$  on IGF-I receptor-mediated signals provide a novel insight into a Jak-independent IL-6 signaling mechanism of receptor cross talk in human myeloma cells. Furthermore, the signaling cross talk between the cytokine receptor, IL-6R $\alpha$ /gp130 and the growth factor receptor tyrosine kinase, fibroblast growth factor receptor (FGFR) 3 appears in myeloma cells carrying t(4;14)(p16.3;q32). In this review we propose several mechanisms of the IL-6-induced cell proliferation that is strictly dependent upon the cellular context in myelomas. [*J Clin Exp Hematopathol* 46(2) : 55-66, 2006]

**Keywords:** CD45, interleukin-6 receptor (IL-6R), myeloma, proliferation, receptor tyrosine kinases (RTKs)

## INTRODUCTION

Immune and hematopoietic systems are regulated through the control of a number of cellular processes including cell growth, differentiation, migration and death by cell-cell interaction and by soluble factors. Ligation of receptors by their specific ligands initiates a signal transduction cascade that leads to multiple biological responses. Intracellular signal transduction events depend on the subtle balance between protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPases) that control tyrosine phosphorylation. In

the immune system, precise and coordinated regulation of this equilibrium allows for rapid responses to foreign antigens, whereas an imbalance between PTKs and PTPases can have pathologic consequences including autoimmunity, immunodeficiency and malignancy<sup>1</sup>.

Cytokines exert multiple biological functions through interactions with their specific receptors. The binding of cytokines to their receptors induces dimerization of the receptors, and triggers activation of intracellular signaling cascades<sup>2</sup>. The earliest event is activation of Janus kinase (Jak) family members including Jak1, Jak2, Jak3 and Tyk2<sup>3</sup>. Activated Jaks phosphorylate a latent cytoplasmic transcription factor, signal transducer and activator of transcription (STAT), the most important substrate for Jaks, initiating the signal transduction pathways critical for cytokine effects<sup>4</sup>. Seven members of STAT family proteins are activated by distinct cytokines, and therefore, the Jak-STAT pathway seems to be a key pathway to introduce the specific cellular responses by cytokines. Eight members of suppressor of

Received : Dec 16, 2005

Accepted : Dec 18, 2005

Department of Bio-Signal Analysis, Applied Medical Engineering Science, Graduate School of Medicine, Yamaguchi University, Yamaguchi, Japan

Address correspondence and reprint request to Hideaki Ishikawa, Laboratory of Cellular Signal Analysis, Department of Bio-Signal Analysis, Applied Medical Engineering Science (AMES), Graduate School of Medicine, Yamaguchi University, 1-1-1 Minami-kogushi, Ube, Yamaguchi 755-8505, Japan

cytokine signaling (SOCS) family proteins induced by STATs act in a negative feedback loop to suppress the Jak-STAT pathway via interacting with either Jaks<sup>5,6</sup> or cytokine receptors<sup>7</sup>, resulting in attenuation of the cytokine signal transduction.

Multiple myeloma (MM) is characterized as a clonal human plasma cell-neoplasm, and develops mainly in bone marrow (BM). Human myeloma cells freshly isolated from BM could respond to a cytokine, interleukin-6 (IL-6), and proliferate *in vitro*<sup>8</sup>. However, the only minor populations of myeloma cells show a significant proliferative activity in response to IL-6. Despite the clonal origin of tumor cells, there are phenotypically mixed subpopulations of myeloma cells from the same patient. Immature myeloma cells proliferate markedly in response to IL-6, whereas mature myeloma cells fail to proliferate but secrete antibodies *in vitro*<sup>9</sup>. Thus, myeloma cells are heterogeneous with respect to their biological characters as well as to their immunophenotypes, and we find that CD45-expressing immature cells belong to the proliferating populations in MM<sup>10</sup>.

Some growth factors, such as insulin<sup>11</sup>, insulin-like growth factor-I (IGF-I)<sup>12,13</sup> and basic fibroblast growth factor (bFGF/FGF-2)<sup>14</sup> of which receptors have tyrosine kinase domains are also reported to function as growth and survival factors for MM. Most receptor tyrosine kinases (RTKs) are composed of a single polypeptide chain, and are monomeric in the absence of their ligands. Ligand binding to the extracellular portion of RTKs leads to dimerization of receptors, resulting in autophosphorylation of specific tyrosine residues in the cytoplasmic portion<sup>15</sup>. Tyrosine autophosphorylation either stimulates the intrinsic catalytic (kinase) activity of the receptor or generates recruitment sites for the downstream signaling proteins containing phosphotyrosine-recognition domains, such as src homology (SH) 2 domain or phosphotyrosine-binding domain<sup>16</sup>.

Cytokines and growth factors bind to their receptors on the cell surface and activate signal transduction pathways that frequently lead to cell proliferation and differentiation. Promotion of cell proliferation by cytokines or growth factors has been shown to correlate with the intracellular activation of common protein kinase cascades. Myeloma cells use cytokine receptors, such as IL-6 receptors, lacking kinase activity as main receptors for promoting their growth, while they also express RTKs, such as IGF-I receptors, as well as other type of receptors. In this review, we propose the molecular mechanisms of the IL-6-induced proliferation of myeloma cells, such as the cooperation of several transmembrane molecules with the IL-6 receptor (IL-6R) complexes, that eventually influences the outcome of cellular responses.

## IL-6 AS A GROWTH FACTOR FOR HUMAN MYELOMA CELLS

Among the numerous number of growth enhancing factors for myelomas postulated, such as IGF-I<sup>13</sup>, IL-10<sup>17</sup> and IL-21<sup>18</sup>, IL-6 is considered as the most important and best characterized growth stimulatory factor for myeloma cells. IL-6 has a variety of biological functions in different cells<sup>19</sup>, whereas IL-6 enhances proliferation of myeloma cells which is inhibited by treatment with anti-IL-6 antibodies *in vitro*<sup>8</sup> and *in vivo*<sup>20</sup>. Moreover, in Balb/c mice the overexpression of IL-6 transgene induces plasmacytomas<sup>21</sup>, and the IL-6-deficient mice with Balb/c genetic background are completely resistant to the development of plasmacytomas induced by pristane oil<sup>22</sup> and by myc/raf-expressing retrovirus (J3V1) infection in combination with pristane<sup>23</sup>, demonstrating that IL-6 also plays a crucial pathogenic role in the onset of plasma cell tumors *in vivo*. In addition, IL-6 has an anti-apoptotic effect on myeloma cells<sup>24</sup>, and therefore, IL-6 supports the survival and expansion of myeloma cells not only by stimulating cell division, but also by preventing apoptosis. IL-6 is a member of the IL-6 family of cytokines including leukemia inhibitory factor, oncostatin M, IL-11, ciliary neurotrophic factor and cardiotrophin-1 which commonly use gp130 as a signal transducing molecule (see below), and some are reported to actually stimulate myeloma cell growth<sup>25</sup>. In myeloma IL-6 is provided by the tumor itself<sup>8</sup> and BM stromal cells<sup>26</sup>.

## IL-6 SIGNALING PATHWAYS

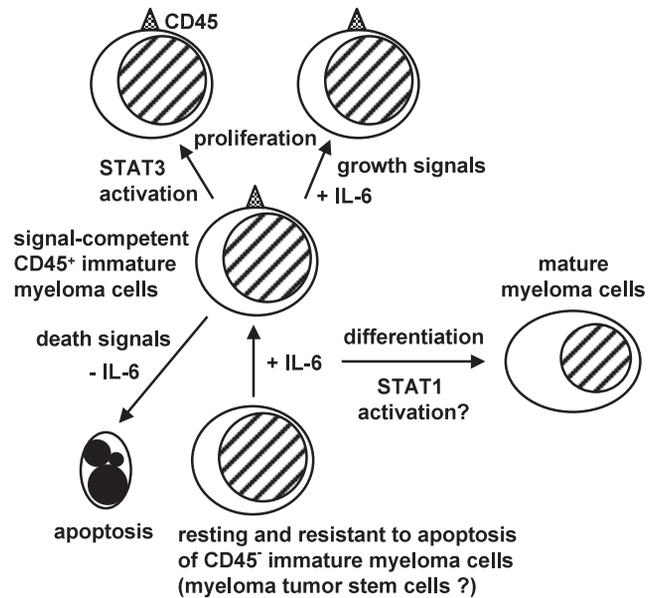
IL-6R complexes consist of two subunits termed as gp80/ $\alpha$  chain and gp130/ $\beta$  chain. IL-6R $\alpha$  directly binds to IL-6, while gp130 responsible for signal transduction is commonly used by the members of the IL-6 family of cytokines<sup>27</sup>. Upon IL-6 binding to IL-6R $\alpha$ , Jaks, such as Jak1 and Tyk2, constitutively associated with gp130 are activated, followed by phosphorylation of the cytoplasmic region of gp130 with the activated Jaks. The phosphorylated tyrosine residues in gp130 recruit STAT3 followed by tyrosine phosphorylation with Jaks. The activated STAT3 dimerizes and translocates to the nucleus in which STAT3 controls the gene expression<sup>28</sup>. Serine phosphorylation of STAT3 is also important for full activation of transcriptional activity<sup>29</sup>. In addition to the Jak-STAT pathway, a simple signaling cascade that directly links the cell membrane and the nucleus, IL-6 also induces the activation of Ras via Sos and Grb2 interacting with SHP-2 that is also recruited to the phosphorylated gp130. Subsequently, the downstream signaling intermediates of Ras, such as Raf-1, MAP or ERK kinase (MEK) 1/2 and extracellular signal-regulated kinase (ERK) 1/2, are activated<sup>30</sup>. Activation of both the Jak-STAT and Ras-mitogen activated protein kinase (MAPK) pathways is neces-

sary for cell proliferation<sup>30</sup>. In some cases, the phosphatidylinositol (PI)-3 kinase pathway is also activated via gp130 where Gab-1 acts as an adaptor molecule in transmitting signals to the PI-3 kinase from gp130 and SHP-2<sup>31</sup>.

## CD45 EXPRESSION, ISOFORMS AND PROLIFERATION OF MYELOMA CELLS

Despite the character of CD45 as a leukocyte common antigen, it has been well known that some acute lymphocytic leukemia (ALL) and plasma cell lines lack the CD45 expression. In looking at human myeloma cells from the MM patients, it appears that there is the CD45 expression on immature myeloma cells but not on mature ones<sup>32-35</sup>. The more careful investigation show that the CD45 molecule is expressed on all nucleated hematopoietic cells including plasma cells, whereas the expression of CD45 molecule in primary myeloma cells and cell lines is quite variable, consistent with the heterogeneity of myeloma cells. In human, CD45 is composed of several isoforms that vary in their extracellular region as a result of tissue or cell type-specific alternative splicing of exons 4, 5, and 6<sup>36</sup>. Although the requirement of the intracellular PTPase domain of CD45 has been documented<sup>37</sup>, the function of the extracellular domain that differs among these isoforms remains a major unresolved issue. B lymphocytes and plasma cells express CD45 and CD45RA (high-molecular weight isoforms), CD45RB but not CD45RO, whereas the restricted subpopulation of myeloma cells and some myeloma cell lines expresses CD45 and CD45RO, CD45RB but not CD45RA<sup>10</sup>, although most myeloma cells lose CD45 expression. It appears that isoforms of CD45 alter during oncogenic event, such as from CD45RA to CD45RO, and CD45RO but not CD45RA seem to have an ability to help the cell proliferation by IL-6 (see below)<sup>38</sup> as some reports have shown functional differences among isoforms<sup>39-42</sup>.

Immature myeloma cells but not mature ones include proliferating cells evaluated by expression of Ki-67 antigen<sup>10</sup>, consistent with the results obtained from [<sup>3</sup>H]thymidine incorporation and cyclin D1<sup>+</sup>p16<sup>-</sup> cell populations<sup>43</sup>. Ki-67<sup>+</sup> myeloma cells are found in the CD45<sup>+</sup> rather than CD45<sup>-</sup> fractions. Thus, the CD45<sup>+</sup> immature myeloma cells appear proliferative populations in MM<sup>10</sup>. Conversely, in the culture without IL-6 those CD45<sup>+</sup> immature myeloma cells undergo apoptosis more readily than CD45<sup>-</sup> immature ones<sup>10</sup>, suggesting that the CD45<sup>+</sup> immature myeloma cells essentially require IL-6, whereas CD45<sup>-</sup> immature ones seem to be more independent of IL-6 (Fig. 1). Despite the proliferating populations of CD45<sup>+</sup> immature myeloma cells, the limiting amounts of IL-6 provided in BM may result in the few numbers of CD45<sup>+</sup> myeloma cells in physiological conditions.



**Fig. 1.** CD45 expression and IL-6 responses in myeloma cells. In immature myeloma cells, CD45<sup>+</sup> cells respond to stimuli including proliferation, differentiation and apoptotic signals, whereas CD45<sup>-</sup> cells are resting and not competent for such signals, thereby resistant to cell growth and death<sup>56</sup>. CD45<sup>-</sup> MPC1-CD49e<sup>-</sup> immature myeloma cells are postulated to potentially belong to a category of tumor stem cells in MM<sup>10</sup>. CD45<sup>-</sup> immature myeloma cells would undergo apoptosis in IL-6-insufficient physiological conditions.

## REQUIREMENT OF SRC FAMILY KINASE ACTIVITY ASSOCIATED WITH CD45 FOR MYELOMA CELL PROLIFERATION BY IL-6

CD45, a receptor-type PTPase, is ubiquitously expressed in all nucleated hematopoietic cells<sup>36</sup>. Genetic defects of CD45 in mice<sup>44</sup> and humans<sup>45</sup> cause severely combined immunodeficiency that demonstrates the essential role of CD45 in the immune system especially for the activation and development of lymphocytes. CD45 regulates tyrosine phosphorylation of the molecules, such as src family PTKs, in the signal transduction of the T cell antigen receptor (TCR) and B cell antigen receptor (BCR)<sup>36,37,44-47</sup>. One conventional model holds that when src family PTKs are phosphorylated at the negative regulatory COOH-terminal tyrosine residue by C-terminal src kinase (Csk), they adopt to the aforementioned closed and inactive conformation. The dominant role of CD45 is to dephosphorylate this negative regulatory tyrosine, setting the stage for antigen receptor signaling to activate src family PTKs by autophosphorylation<sup>37,48-50</sup>.

We find that IL-6 rapidly activates both STAT3 and ERK1/2 in CD45<sup>-</sup> myeloma cell lines, although they fail to proliferate in response to IL-6. These myeloma cell lines lack CD45 expression and src family PTK activation, implying

that in myeloma cells the activation of STAT3 and ERK1/2 is not sufficient for cell proliferation enhanced by IL-6. The activation of src family PTKs associated with the CD45 PTPase seem to be a prerequisite for the myeloma cell proliferation that further requires the activation of STAT3 and ERK1/2 by IL-6<sup>51</sup>. Among the molecules we tested, only Lyn appears to interact with CD45 in CD45<sup>+</sup> myeloma cell lines, results suggesting that Lyn could be a major molecule regulated by the CD45 PTPase in myelomas. Indeed, inhibition of either CD45 PTPase or src family PTKs suppresses IL-6-enhanced DNA synthesis of CD45<sup>+</sup> rather than CD45<sup>-</sup> myeloma cells. Thus, with respect to the IL-6-induced proliferation of myeloma cells, CD45 could act as a positive regulator by controlling src family PTK activity, similarly to mitogenic signals via BCR on B cells.

Although the src family PTKs have been shown to activate STATs<sup>52,53</sup>, the inhibition of Lyn expression or kinase activity does not influence STAT3 activation in CD45<sup>+</sup> myeloma cells. Thus, in myeloma cells src family PTKs are not directly involved in STAT3 and Ras-ERK1/2 activation by IL-6. Recent reports have supported our suggestion, showing that Lyn is required for granulocyte-colony stimulating factor (G-CSF)-induced cell proliferation<sup>54</sup>, and that the G-CSF-stimulated signals require src family PTK activities dissociated from the Jak-STAT pathway<sup>55</sup>. The src family PTKs may phosphorylate several molecules including adaptor molecules, enzymes, structural proteins and transcription factors, resulting in a diversification of the initial signals<sup>47</sup>. We also find that in CD45<sup>+</sup> myeloma cells Lyn kinase is likely to activate phospholipase C (PLC)- $\gamma$ -mediated signals, such as

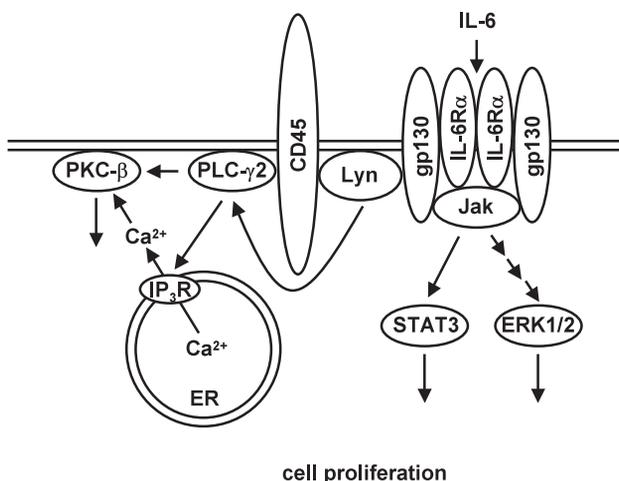
cellular calcium influxes and protein kinase C (PKC) activation, independent of STAT3 and ERK1/2 pathways (Fig. 2)<sup>56</sup>.

## IL-6-INDUCED TRANSLOCATION OF CD45 TO LIPID RAFTS IN MYELOMA CELLS

Considering the relationship between CD45 and its substrates, the cellular localization of CD45 and its accessibility to the substrates may define the activity on signaling. Lipid raft microdomains represent cholesterol- and glycosphingolipid-enriched dynamic patches in the plasma membrane and organize the plasma membrane into functional units<sup>57</sup>. These raft domains act as platforms for conducting a variety of cellular functions including TCR, BCR and cytokine signaling<sup>58-60</sup>. Several protein families have been reported to modify lipid rafts structurally and functionally that include membrane integral proteins, such as caveolins and flotillins, and lipid chain-modified proteins, such as src family PTKs and Ras<sup>61</sup>.

We find that CD45RO and CD45RB but not CD45RA are translocated to lipid rafts in myeloma cell lines and an IL-6R $\alpha$ -transfected B cell line in response to IL-6. Furthermore, IL-6 induces a more rapid translocation of CD45RO to lipid rafts than CD45RB, and the translocated CD45 functions as a positive regulator of proliferation signaling by triggering dephosphorylation of Lyn Tyr<sup>507</sup> on lipid rafts, where IL-6R $\alpha$ , gp130 and Lyn are constitutively present<sup>38</sup>. Glycosylation of CD45 is reported to regulate the formation of clusters induced by galectin-1 binding and resultant phosphatase activity<sup>62</sup>. Also, we cannot exclude the possibility of a different complex with other proteins, different substrate binding or different type of dimeric formation.

As for the functional role of the translocated CD45 in myeloma cells with IL-6 stimulation, we show the formation of a complex between CD45 and Lyn, decreased phosphorylation of Lyn Tyr<sup>507</sup> at the carboxy terminal and subsequent kinase domain Tyr<sup>396</sup> phosphorylation by IL-6. Csk is recruited by phosphoprotein-associated with glycosphingolipid-enriched microdomains (PAG) which targets lipid rafts by palmitoylation<sup>63,64</sup>. The association of PAG with Csk is controlled by dephosphorylation of PAG. In myeloma cells PAG dephosphorylation and the resultant release of Csk from lipid rafts occurs after IL-6 stimulation in accordance with CD45 translocation. CD45 might be a candidate for the PTPase of PAG and dephosphorylation of PAG allows Csk to be released from PAG<sup>65</sup>. We show that CD45 is directly associated with PAG in lipid rafts after IL-6 stimulation. Thus, IL-6 treatment induces the translocation of CD45 to lipid rafts sequentially followed by the association of CD45 with Lyn and PAG, dephosphorylation of Lyn Tyr<sup>507</sup> and PAG Tyr<sup>314</sup>, Lyn activation and Csk release from lipid rafts. Again, CD45 functions as a positive regulator of signal transduction in myeloma cells, although CD45 is also reported to negatively



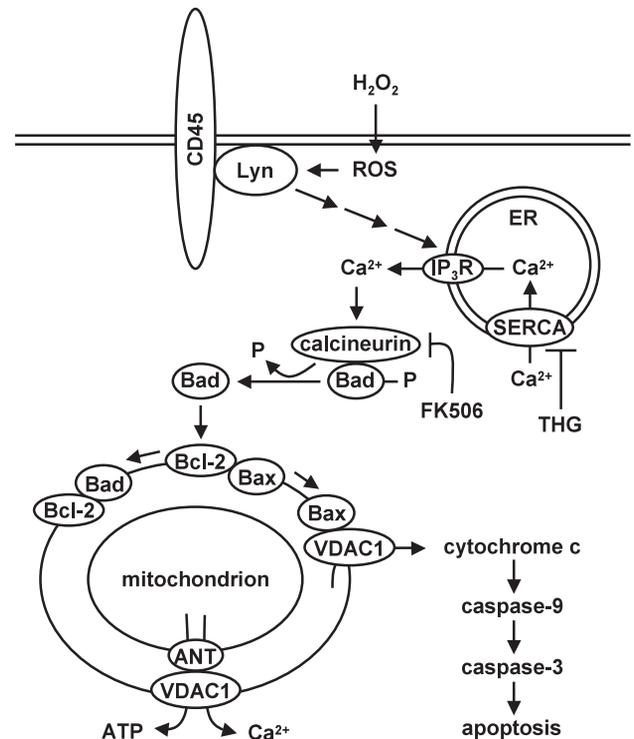
**Fig. 2.** CD45 plays a pivotal role in the IL-6-induced growth promotion of myeloma cells. The activation of Lyn associated with CD45 in parallel with STAT3 and ERK1/2 activation is induced by IL-6 in myeloma cells, followed by the activation of PLC- $\gamma$ 2, PKC and Ca<sup>2+</sup> influxes that are essential for the IL-6-induced proliferation of tumor cells<sup>51,56</sup>. IP<sub>3</sub>R: inositol 1, 4, 5-triphosphate (IP<sub>3</sub>) receptor.

regulate cytokine signaling<sup>66</sup>. We also find that the translocated CD45 binds to flotillin 2, which possibly organizes the network of lipid rafts. The function of flotillin and the significance of the complex with CD45 is not clear, however, the report that flotillins form preassembled platforms in hematopoietic cells and these platforms recruit signaling molecules upon activation through lipid rafts<sup>67</sup> raises the possibility that flotillins might participate in CD45-recruiting mechanisms to lipid rafts to transduce IL-6 signal from IL-6R $\alpha$ .

### CD45<sup>+</sup> MYELOMA CELLS ARE SUSCEPTIBLE TO APOPTOSIS

As cell viability of CD45<sup>-</sup> myeloma cells *in vitro* is better than CD45<sup>+</sup> myeloma cells freshly isolated from MM patients<sup>10</sup>, we show that CD45<sup>+</sup> myeloma cell lines are more sensitive to several apoptotic stimuli as well as a mitogenic stimulus<sup>56</sup>. The intracellular reactive oxygen species (ROS) and calcium ion (Ca<sup>2+</sup>) seem to initiate the cellular events, such as the activation of the src family PTKs and calcineurin phosphatase, which trigger the apoptosis upon the oxidative or endoplasmic reticulum (ER) stresses. The CD45 expression is likely to enhance the hydrogen peroxide-induced apoptosis via the activation of the src family PTKs that is regulated by their redox state<sup>68-72</sup>. In contrast, the thapsigargin-induced apoptosis is related to the increased expression of voltage-dependent anion channel (VDAC) 1 that is identified as a gene highly expressed in a CD45<sup>+</sup> myeloma cell line by cDNA subtraction. Bad sequestered by calcineurin in cytosol moved to mitochondria and bound to Bcl-2 in response to the increase in the intracellular Ca<sup>2+</sup> concentration, resulting in the enhanced release of cytochrome c presumably through the highly expressed VDAC1 associated with Bax (Fig. 3). The VDAC protein is a subunit of the mitochondrial permeability transition pore (PTP), a large channel whose opening results in rapid loss of membrane potential and organellar swelling, quickly leading to cytochrome c release and apoptotic cell death<sup>73</sup>. Bax and Bak enlarge the VDAC channel which is contrarily closed by Bcl-xL<sup>74</sup>. VDAC1 plays an essential role in apoptosis in mammalian cells<sup>75</sup>, and the increase in VDAC expression may facilitate the release of cytochrome c during apoptosis<sup>76</sup>.

We show that CD45<sup>+</sup> myeloma cells accompanied by the increased expression of VDAC1 seem to be sensitive to the various apoptotic stimuli, such as serum deprivation, ultraviolet-ray irradiation, heat shock, SERCA inhibitor (thapsigargin), hydrogen peroxide, arsenic trioxide, interferon (IFN)- $\gamma$  and melphalan treatments<sup>56</sup>. We find the association of calcineurin with Bad in CD45<sup>+</sup> myeloma cells more than CD45<sup>-</sup> ones. The mitochondrial translocation of Bad and the increased association of Bad with Bcl-2 are also observed in CD45<sup>+</sup> myeloma cells treated with hydrogen peroxide or thapsigargin. The oxidative stress-induced activation of the src



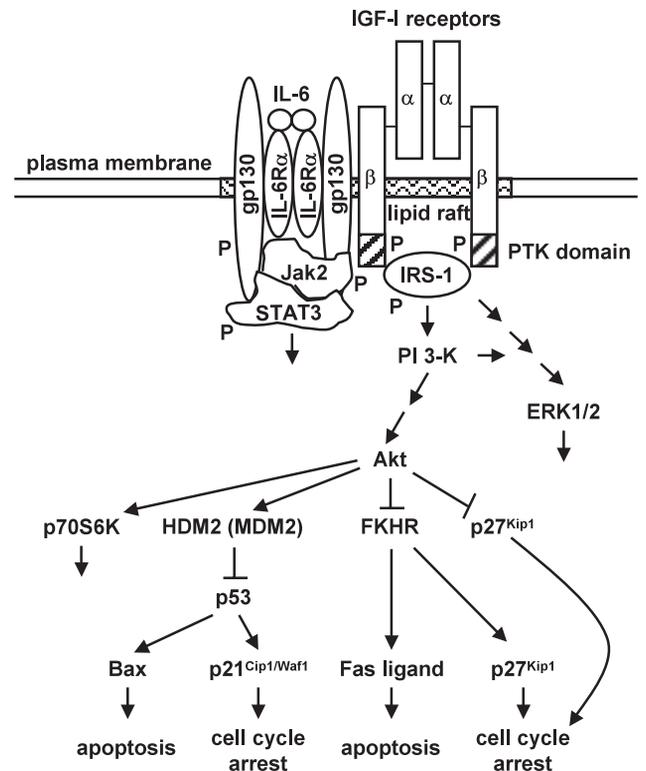
**Fig. 3.** The mitochondrial apoptosis pathways in CD45<sup>+</sup> myeloma cells accompanied by the increased expression of VDAC1. The src family kinases may be activated by the intracellular ROS in CD45<sup>+</sup> myeloma cells, followed by Ca<sup>2+</sup> influxes, calcineurin activation, mitochondrial translocation of Bad, dysfunction of Bcl-2 and cytochrome c release presumably through the increased expression of VDAC1. Bax released from Bcl-2 interacts with VDAC1, leading to cytochrome c release and apoptotic cell death<sup>56</sup>. H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide; THG: thapsigargin; SERCA: sarcoplasmic-endoplasmic reticulum Ca<sup>2+</sup> adenosine triphosphatases; ANT: adenine nucleotide translocator.

family PTKs in CD45<sup>+</sup> myeloma cells leads to the intracellular Ca<sup>2+</sup> fluxes, and the sustained increase in cytosolic Ca<sup>2+</sup> leads to the activation of the serine/threonine phosphatase, calcineurin. Bad dephosphorylated by calcineurin is considered to move to mitochondria and acts as an antagonist for Bcl-2/Bcl-xL, and the dysfunction of Bcl-2 that precedes the cytochrome c release through the VDAC1 associated with Bax leads to apoptosis of the cells (Fig. 3)<sup>77</sup>. The CD45 expression renders myeloma cells competent for several apoptotic stimuli as well as the mitogenic stimulus, resulting in either proliferation or apoptosis of CD45<sup>+</sup> myeloma cells dependently upon the kinds of stimuli (Fig. 1). Thus, CD45 may define the signaling thresholds that are critical for the IL-6-induced proliferation and the oxidative stress-induced apoptosis of human myeloma cells.

## RECEPTOR CROSS TALK BETWEEN IL-6 AND IGF-I IN MYELOMA CELLS

Numerous biological responses of different cell types are induced by IL-6 which activates STAT3 and Ras-ERK1/2 via Jaks, and the balance of activation of both pathways are considered to direct the cell fate in response to IL-6<sup>78</sup>. IL-6 is the most potent growth factor for myelomas *in vitro* and *in vivo*, while IGF-I, a member of so-called growth factors whose receptors possess the tyrosine kinase domains is a potent growth and survival factor for wide variety of cells. We find that IGF-I receptors are phosphorylated by IL-6 stimulation in IL-6R $\alpha$  highly expressing myeloma cell lines. The mechanism appears to be the complex formation of IL-6R $\alpha$  with IGF-I receptor  $\beta$  by IL-6 stimulation whereas it is likely to be independent of Jaks. In addition to STAT3 and ERK1/2 pathways, IL-6 activates Akt downstream of PI-3 kinase via phosphorylation of IGF-I receptors, and the PI-3 kinase-Akt pathway is further necessary for IL-6-promoted proliferation of myeloma cells. The activated Akt could contribute to anti-apoptosis of cells presumably due to inactivation of forkhead transcription factor (FKHR), p27<sup>Kip1</sup> and p53. Hence, in CD45<sup>-</sup> myeloma cell lines, the IL-6-induced activation of STAT3 and ERK1/2 is important but not sufficient for cell survival and proliferation that further require the activation of PI-3 kinase-Akt-mediated pathways. Accordingly, under the cellular context of the elevated expression of IL-6R $\alpha$  on myeloma cells, it is assumed that IL-6R $\alpha$  molecules are located close to IGF-I receptors at lipid rafts, and IL-6 stimulation triggers the complex formation of IL-6R $\alpha$  not only with gp130 but also with IGF-I receptors, leading to autophosphorylation of IGF-I receptor  $\beta$  and subsequent activation of PI-3 kinase-Akt pathways (Fig. 4)<sup>79</sup>. Although the cross talk of signals mediated by a cytokine and growth factor has been previously reported in the case of the phosphorylation of EGF receptors by the growth hormone-activated Jak2<sup>80</sup>, a Jak2 inhibitor could not suppress the phosphorylation of IGF-I receptor  $\beta$  in myeloma cells, suggesting that the IL-6-induced activation of Jak2 is not involved in the activation of IGF-I receptor-mediated signals. Gab-1, insulin receptor substrate (IRS)-1 and IRS-2 could not be co-precipitated with gp130 in myeloma cells, also supporting that the activation of PI-3 kinase and Akt resulted from the phosphorylation of IGF-I receptors.

Furthermore, our finding of colocalization of IL-6R $\alpha$  and IGF-I receptors at lipid rafts in myeloma cells is consistent with a recent report which reveals the blockade of IL-6 and IGF-I signaling by a raft inhibitor<sup>81</sup>. These suggest that IL-6 receptors and IGF-I receptors seem to be closely linked together, and therefore the highly expressed IL-6R $\alpha$  likely enhances the accessibility of IL-6R $\alpha$  with IGF-I receptor  $\beta$  on cell surface membranes. Cross talk of IFN- $\alpha/\beta$  signals with gp130 has also been reported that IFNAR-1 and gp130 exist



**Fig. 4.** Synergy of IL-6 and IGF-I receptors in myeloma cells. IL-6 induces the activation of the PI-3 kinase signaling pathways that include Akt, p70S6 kinase and FKHR in myeloma cells that highly express IL-6 receptor  $\alpha$ . The activated Akt enhances the cytoplasmic accumulation of p27<sup>Kip1</sup> and p53 proteins, and blocks the expression of Fas ligand, p27<sup>Kip1</sup>, p21<sup>Cip1/Waf1</sup> and bax genes. IL-6 stimulation induces co-localization of IL-6R $\alpha$  and IGF-I receptors at lipid rafts that leads to the IGF-I-independent tyrosine phosphorylation of IGF-I receptor  $\beta$ <sup>79</sup>. MDM2: mouse double minute 2; HDM2: human MDM2; PI 3-K: PI-3 kinase; p70S6K: p70S6-kinase.

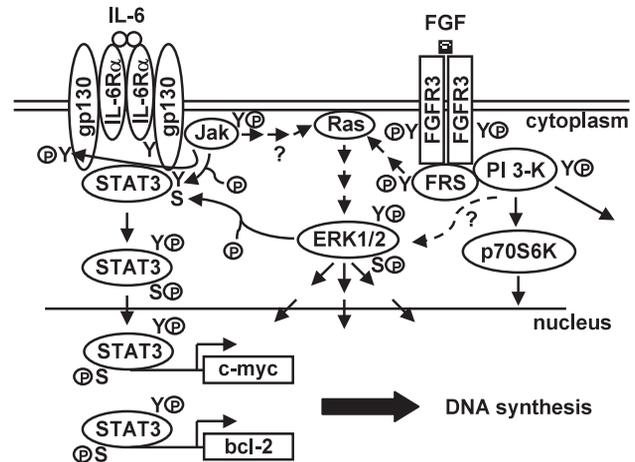
in close proximity<sup>82</sup>. The results suggest the assembly of cytokine receptor subunits, which may represent a 'receptosome'-like structure, allowing the unique signaling cross talks to occur<sup>82</sup>. Taken together, it is tempting to speculate that the elevated expression of IL-6R $\alpha$  could increase the frequency of complex formation of IL-6R $\alpha$  with IGF-I receptors at lipid rafts in response to IL-6, presumably resulting in the altered conformation of IGF-I receptors that triggers their autophosphorylation independently of both Jaks and IGF-I. The increased IL-6R $\alpha$  could interact with IGF-I receptors at lipid rafts in response to IL-6 and induce signal divergence from IL-6 receptor complexes to downstream interacting receptors. This, therefore, provides a novel insight into the IL-6-induced growth mechanism of IL-6R $\alpha$  highly expressing myeloma cells of the Jak-independent synergy between IL-6 receptors and IGF-I receptors.

## SIGNALING CROSS TALK OF IL-6R WITH FGFR3 IN MYELOMA CELLS

Chromosomal translocations involving immunoglobulin (Ig) loci are found in B-cell neoplasms including MM<sup>83</sup>. Most cases of myeloma exhibit Ig translocations involving numerous partner chromosomes, and may provide an early immortalizing event. Many genes are involved in these translocations that occur to the IgH S regions. Some partner genes appear to account for the majority of primary IgH (14q32) translocations, such as 4p16 (FGF receptor 3; FGFR3 and multiple myeloma SET domain; MMSET/Wolf-Hirschhorn syndrome candidate 1; WHSC1)<sup>84-88</sup>, 6p25 (IFN regulatory factor-4)<sup>89</sup>, 11q13 (cyclin D1)<sup>86</sup>, and 16q23 (c-maf)<sup>90</sup>. The chromosomal translocation, t(4;14)(p16;q32) results in ectopic expression of FGFR3 and IgH/MMSET hybrid transcripts in myeloma cells due to the effects of the 3' IgH enhancer<sup>84,85,87,88</sup>. Despite the expression of FGFR3 and IL-6R $\alpha$  on a human myeloma cell line, KMS-11 carrying t(4;14)(p16.3;q32)<sup>84,85</sup>, its proliferation is not augmented by bFGF/FGF-2, aFGF/FGF-1 or IL-6 alone, whereas IL-6 together with FGF accelerates the proliferation, indicating the biological synergy between IL-6 and FGF in KMS-11<sup>91</sup>.

Phosphorylation of a tyrosine residue at 705 in STAT3 is observed in KMS-11 in response to IL-6. In contrast, bFGF induces the activation of ERK1/2 and PI-3 kinase. These results indicate that IL-6 and bFGF activate the distinct intracellular signaling pathways, and both the ERK1/2 and PI-3 kinase pathways activated by bFGF are necessary for the enhanced proliferation of KMS-11. bFGF but not IL-6 stimulation induces the phosphorylation of Ser727 in STAT3, and a selective MEK1/2 inhibitor abolishes the serine- but not tyrosine-phosphorylation of STAT3 in response to bFGF and IL-6 stimulations, supporting the notion that two independent signaling pathways converge on STAT3 to regulate its function in the cell. Thus, the bFGF-induced activation of ERK1/2 pathway is involved in the phosphorylation of the critical serine residue of STAT3<sup>92</sup>. Furthermore, the bFGF-induced activation of ERK1/2 seems to enhance the transcriptional activity of STAT3. Co-stimulation of KMS-11 with bFGF and IL-6 leads to marked expression of STAT3 target genes, such as c-myc and bcl-2, further suggesting the relevance of STAT3 phosphorylated at both Tyr705 and Ser727 for the full activation as a transcription factor (Fig. 5). In addition, the expression of FGFR3 may render the IL-6-responsive CD45<sup>+</sup> myeloma cells sensitive to IL-6 for cell growth because the suboptimal doses of IL-6 enhance the proliferation of the FGFR3-transfected CD45<sup>+</sup> myeloma cell line. This may be physiologically important because unlike *in vitro* experiments, the amounts of soluble factors, such as IL-6 and FGF, must be limited *in vivo*.

Ig translocations involve a plethora of different molecules that influence many different pathways. We show the

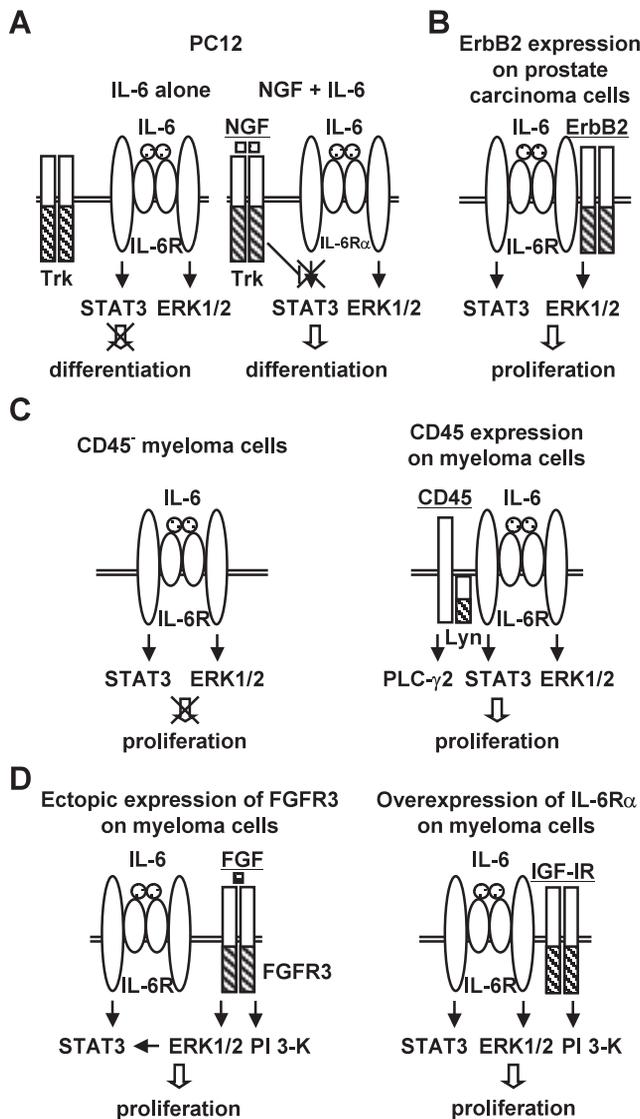


**Fig. 5.** Enhanced proliferation of myeloma cells by interleukin-6 cooperating with FGFR3-mediated signals. IL-6 and FGF induce the distinct intracellular signaling pathways in myeloma cells carrying t(4;14)(p16.3;q32) that ectopically express FGFR3. FGF and IL-6 activate ERK1/2, PI-3 kinase and STAT3, respectively. The serine phosphorylation of STAT3 via the FGF-induced ERK1/2 activation contributes to the full activation of STAT3, leading to the expression of c-myc and bcl-2 genes and cell proliferation<sup>91</sup>. FRS: FGF receptor substrate.

proliferative synergy between IL-6 and FGF, and explore the signaling events underlying the important biologic interaction between IL-6 and FGF in t(4;14)(p16.3;q32) MM. In agreement with synergistic effect of cytokines and growth factors on cells through complementary signaling pathways<sup>93</sup>, we provide a novel mechanism of MM growth mediated by signaling cross talk between RTK and cytokine receptor.

## CONCLUSION

In this review, we discuss that the activities of src family PTKs associated with the CD45 PTPase seem to be a prerequisite for the myeloma cell proliferation that further requires the activation of STAT3 and ERK1/2 by IL-6 (Fig. 2 and 6)<sup>51</sup>. Although the regulatory mechanisms of the CD45 PTPase activity still remain unclear in myeloma cells, the tight control of CD45 isoform expression supports the possibility that differential isoform expression may regulate CD45 homodimerization and function. Even though IL-6 may induce the similar intracellular signals, such as the Jak-STAT and Ras-MAPK pathways in several cell types, the distinct biological responses by IL-6 are observed in different cell types, implicating that cellular context would play a crucial role in the outcome of cytokine-induced cellular responses. Expression of CD45 PTPase would result in the activation of src family PTKs which may amplify the biological response to IL-6 and modify the signaling threshold required for myeloma cell proliferation by IL-6. Altering the biological threshold repre-



sents that cells could differentially respond to IL-6 by using the same signal transduction machinery. Active regulation of src family PTKs by CD45 PTPase may be important for myeloma cells in a primed state, capable of response to IL-6.

It also becomes clear that biological effects of IL-6 could be modified by other factors (Fig. 6). IL-6 can induce neurite outgrowth of PC12 cell line pretreated with nerve growth factor (NGF) which represses the STAT3 activation<sup>94</sup>, and the IL-6-induced proliferation of prostate carcinoma cell line, LNCaP requires ErbB2 that is essential for the ERK1/2 activation<sup>95</sup>. It is also reported that cross talk of bone morphogenetic protein-2 and gp130-mediated signals via p300 in the nucleus of fetal neuronal progenitor cells to induce astrocytes<sup>96</sup>. In myelomas, we also show that the ectopically expressed FGFR3-mediated signals cooperating with gp130-induced signals promote CD45<sup>+</sup> myeloma cell proliferation<sup>91</sup>,

**Fig. 6.** IL-6-mediated signals via gp130 modified or cooperated with other transmembrane molecules.

(A) Neurite outgrowth of PC12 cell line is induced by IL-6 pretreated with NGF but not by IL-6 alone<sup>94</sup>. NGF suppresses the IL-6-induced STAT3 activation by unknown mechanisms. (B) ErbB2 is essential for the proliferation and ERK1/2 activation by IL-6 in prostate carcinoma cell line, LNCaP<sup>95</sup>. (C) Activation of STAT3 and ERK1/2 is not sufficient for the IL-6-induced proliferation of myeloma cells that further requires Lyn activity associated with CD45 expression in myeloma cell lines<sup>38, 51</sup>. (D) In CD45<sup>+</sup> myeloma cell line, KMS-11 gp130-mediated signals cooperated with signals via ectopically expressed FGFR3 enhance the proliferation, where ERK1/2 activated by FGF stimulation phosphorylates a critical serine residue of STAT3, resulting in the full activation of STAT3<sup>91</sup>. In another CD45<sup>+</sup> myeloma cell line, NOP-2 overexpression of IL-6R $\alpha$  activates IGF-1/insulin receptor systems in response to IL-6 and exerts multiple intracellular signaling cascades that contribute to biological responses<sup>79</sup>. These indicate that the IL-6-induced cellular responses are seriously influenced by signals via the other receptors or by different cellular contexts. Shaded boxes indicate tyrosine kinase activity.

and overexpression of IL-6R $\alpha$  diversifies the IL-6-mediated intracellular signals that seem to be critical for biological responses of CD45<sup>+</sup> myeloma cells<sup>79</sup>. Thus, we propose the proliferative mechanisms of myeloma cells by IL-6 of which receptor-mediated signals modified and influenced by other transmembrane molecule-mediated signals, such as the ERK1/2 activation via FGFR3, and IL-6 receptors cooperate with IGF-1 receptors, leading to the activation of the PI-3 kinase pathways.

## ACKNOWLEDGMENTS

This work was supported by in part grants from the Ministry of Education, Science, Sports and Culture of Japan, the Ministry of Health and Welfare of Japan, the Japan Society for the Promotion of Science, and also by International Myeloma Foundation, Mitsubishi Pharma Research Foundation, Public Trust Leukemia Research Fund, Public Trust Haraguchi Memorial Cancer Research Fund, and Yamanouchi Foundation for Research on Metabolic Disorders.

## REFERENCES

- Li L, Dixon JE: Form, function, and regulation of protein tyrosine phosphatases and their involvement in human diseases. *Semin Immunol* 12: 75-84, 2000
- Kishimoto T, Taga T, Akira S: Cytokine signal transduction. *Cell* 76: 253-262, 1994
- Taniguchi T: Cytokine signal through nonreceptor protein tyrosine kinases. *Science* 268: 251-255, 1995

- 4 Ihle JN : STATs : signal transducers and activators of transcription. *Cell* 84 : 331-334, 1996
- 5 Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H, Miyazaki T, Leonor N, Taniguchi T, Fujita T, Kanakura Y, Komiya S, Yoshimura A : A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* 387 : 921-924, 1997
- 6 Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S, Kishimoto T : Structure and function of a new STAT-induced STAT inhibitor. *Nature* 387 : 924-929, 1997
- 7 Yoshimura A, Ohkubo T, Kiguchi T, Jenkins NA, Gilbert DJ, Copeland NG, Hara T, Miyajima A : A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. *EMBO J* 14 : 2816-2826, 1995
- 8 Kawano M, Hirano T, Matsuda T, Taga T, Horii Y, Iwato K, Asaoku H, Tang B, Tanabe O, Tanaka H, Kuramoto A, Kishimoto T : Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. *Nature* 332 : 83-85, 1988
- 9 Kawano MM, Huang N, Harada H, Harada Y, Sakai A, Tanaka H, Iwato K, Kuramoto A : Identification of immature and mature myeloma cells in the bone marrow of human myeloma. *Blood* 82 : 564-570, 1993
- 10 Fujii R, Ishikawa H, Mahmoud MS, Asaoku H, Kawano MM : MPC-1<sup>-</sup>CD49e<sup>-</sup> immature myeloma cells include CD45<sup>+</sup> subpopulations that can proliferate in response to IL-6 in human myelomas. *Br J Haematol* 105 : 131-140, 1999
- 11 Freund GF, Kulas DF, Monney RA : Insulin and IGF-1 increase mitogenesis and glucose metabolism in the multiple myeloma cell line, RPMI 8226. *J Immunol* 151 : 1811-1820, 1993
- 12 Xu F, Gardner A, Tu Y, Michl P, Prager D, Lichtenstein A : Multiple myeloma cells are protected against dexamethasone induced apoptosis by insulin-like growth factors. *Br J Haematol* 97 : 429-440, 1997
- 13 Georgii-Hemming P, Wiklund HJ, Ljunggren O, Nilsson K : Insulin-like growth factor I is a growth and survival in human multiple myeloma cell line. *Blood* 88 : 2250-2258, 1996
- 14 Plowright EE, Li Z, Bergsagel L, Chesi M, Barber DL, Branch DR, Hawley RG, Stewart AK : Ectopic expression of fibroblast growth factor receptor 3 promotes myeloma cell proliferation and prevents apoptosis. *Blood* 95 : 992-998, 2000
- 15 Urrlich A, Schlessinger J : Signal transduction by receptors with tyrosine kinase activity. *Cell* 61 : 203-212, 1990
- 16 Pawson T : Protein modules and signalling networks. *Nature* 373 : 573-580, 1995
- 17 Lu ZY, Zhang XG, Rodriguez C, Wijdenes J, Gu ZJ, Morel-Fournier B, Harousseau JL, Bataille R, Rossi JF, Klein B : Interleukin-10 is a proliferation factor but not a differentiation factor for human myeloma cells. *Blood* 85 : 2521-2527, 1995
- 18 Brenne A-T, Baede Ro T, Waage A, Sundan A, Borset M, Hjorth-Hansen H : Interleukin-21 is a growth and survival factor for human myeloma cells. *Blood* 99 : 3756-3762, 2002
- 19 Akira S, Taga T, Kishimoto T : Interleukin-6 in biology and medicine. *Adv Immunol* 54 : 1-78, 1993
- 20 Zhang XG, Bataille R, Wijdenes J, Klein B : Interleukin-6 dependence of advanced malignant plasma cell dyscrasia. *Cancer* 69 : 1373-1376, 1992
- 21 Suematsu S, Matsusaka T, Matsuda T, Ohno S, Miyazaki J, Yamamura K, Hirano T, Kishimoto T : Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin 6 transgenic mice. *Proc Natl Acad Sci USA* 89 : 232-235, 1992
- 22 Lattanzio G, Libert C, Aquilina M, Cappelletti M, Ciliberto G, Musiani P, Poli V : Defective development of pristane-oil-induced plasmacytomas in interleukin-6-deficient BALB/c mice. *Am J Pathol* 151 : 689-696, 1997
- 23 Hilbert DM, Kopf M, Mock BA, Kohler G, Rudikoff S : Interleukin 6 is essential for *in vivo* development of B lineage neoplasmas. *J Exp Med* 182 : 243-248, 1995
- 24 Kawano MM, Mihara K, Huang N, Tsujimoto T, Kuramoto A : Differentiation of early plasma cells on bone marrow stromal cells requires interleukin-6 for escaping from apoptosis. *Blood* 85 : 487-494, 1995
- 25 Zhang XG, Gu JJ, Lu ZY, Yasukawa K, Yancopoulos GD, Turner K, Shoyab M, Taga T, Kishimoto T, Bataille R, Klein B : Ciliary neutropic factor, interleukin 11, leukemia inhibitory factor, and oncostatin M are growth factors for human myeloma cell lines using the interleukin 6 signal transducer gp130. *J Exp Med* 179 : 1337-1342, 1994
- 26 Klein B, Zhang XG, Jourdan M, Content J, Houssiau F, Aarden L, Piechaczyk M, Bataille R : Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. *Blood* 73 : 517-526, 1989
- 27 Hirano T, Matsuda T, Nakajima K : Signal transduction through gp130 that is shared among the receptors for the interleukin 6 related cytokine subfamily. *Stem cells* 12 : 262-277, 1994
- 28 Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Thierfelder WE, Kreider B, Silvennoinen O : Signaling by the cytokine receptor superfamily : JAKs and STATs. *Trends Biochem Sci* 19 : 222-227, 1994
- 29 Wen Z, Zhong Z, Darnell Jr JE : Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. *Cell* 82 : 241-250, 1995
- 30 Fukada T, Hibi M, Yamanaka Y, Takahashi-Tezuka M, Fujitani Y, Yamaguchi T, Nakajima K, Hirano T : Two signals are necessary for cell proliferation induced by a cytokine receptor gp130 : involvement of STAT3 in anti-apoptosis. *Immunity* 5 : 449-460, 1996
- 31 Takahashi-Tezuka M, Yoshida Y, Fukada T, Ohtani T, Yamanaka Y, Nishida K, Nakajima K, Hibi M, Hirano T : Gab1 acts as an adapter molecule linking the cytokine receptor gp130 to ERK mitogen-activated protein kinase. *Mol Cell Biol* 18 : 4109-4117, 1998
- 32 Hata H, Matsuzaki H, Sonoki T, Takemoto S, Kuribayashi N, Nagasaki A, Takatsuki K : Establishment of a CD45-positive immature plasma cell line from an aggressive multiple myeloma with

- high serum lactate dehydrogenase. *Leukemia* 8 : 1768-1773, 1994
- 33 Witzig TE, Kimlinger TK, Ahmann GJ, Katzmann JA, Greipp PR : Detection of myeloma cells in the peripheral blood by flow cytometry. *Cytometry* 26 : 113-120, 1996
- 34 Joshua D, Petersen A, Brown R, Pope B, Snowdon L, Gibson J : The labelling index of primitive plasma cells determines the clinical behaviour of patients with myelomatosis. *Br J Haematol* 94 : 76-81, 1996
- 35 Schneider U, van Lessen A, Huhn D, Serke S : Two subsets of peripheral blood plasma cells defined by differential expression of CD45 antigen. *Br J Haematol* 97 : 56-64, 1997
- 36 Trowbridge IS, Thomas ML : CD45 : an emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. *Annu Rev Immunol* 12 : 85-116, 1994
- 37 Hermiston ML, Xu Z, Weiss A : CD45 : a critical regulator of signaling thresholds in immune cells. *Annu Rev Immunol* 21 : 107-137, 2003
- 38 Li F-J, Tsuyama N, Ishikawa H, Obata M, Abroun S, Liu S, Otsuyama K-I, Zheng X, Ma Z, Maki Y, Kawano MM : A rapid translocation of CD45RO but not CD45RA to lipid rafts in IL-6-induced proliferation in myeloma. *Blood* 105 : 3295-3302, 2005
- 39 Novak TJ, Farber D, Leitenberg D, Hong SC, Johnson P, Bottomly K : Isoforms of the transmembrane tyrosine phosphatase CD45 differentially affect T cell recognition. *Immunity* 1 : 109-119, 1994
- 40 Chui D, Ong CJ, Johnson P, Teh HS, Marth JD : Specific CD45 isoforms differentially regulate T cell receptor signaling. *EMBO J* 13 : 798-807, 1994
- 41 Leitenberg D, Novak TJ, Farber D, Smith BR, Bottomly K : The extracellular domain of CD45 controls association with the CD4-T cell receptor complex and the response to antigen-specific stimulation. *J Exp Med* 183 : 249-259, 1996
- 42 Dorman S, Sebestyen Z, Gamble J, Nagy P, Bodnar A, Alldridge L, Doe S, Holmes N, Goff LK, Beverley P, Szollosi J, Alexander DR : Differential association of CD45 isoforms with CD4 and CD8 regulates the actions of specific pools of p56lck tyrosine kinase in T cell antigen receptor signal transduction. *J Biol Chem* 277 : 1912-1918, 2002
- 43 Kawano MM, Mahmoud MS, Ishikawa H : Cyclin D1 and p16<sup>INK4A</sup> are preferentially expressed in immature and mature myeloma cells, respectively. *Br J Haematol* 99 : 131-138, 1997
- 44 Byth KF, Conroy LA, Howlett S, Smith AJ, May J, Alexander DR, Holmes N : CD45-null transgenic mice reveal a positive regulatory role for CD45 in early thymocyte development, in the selection of CD4<sup>+</sup>CD8<sup>+</sup> thymocytes, and in B cell maturation. *J Exp Med* 183 : 1707-1718, 1996
- 45 Kung C, Pingel JT, Heikinheimo M, Klemola T, Varkila K, Yoo LI, Vuopala K, Poyhonen M, Uhari M, Rogers M, Speck SH, Chatila T, Thomas ML : Mutations in the tyrosine phosphatase CD45 gene in a child with severe combined immunodeficiency disease. *Nat Med* 6 : 343-345, 2000
- 46 Penninger JM, Irie-Sasaki J, Sasaki T, Oliveirados-Santos AJ : CD45 : new jobs for an old acquaintance. *Nat Immunol* 2 : 389-396, 2001
- 47 Corey SJ, Anderson SM : Src-related protein tyrosine kinases in hematopoiesis. *Blood* 93 : 1-14, 1999
- 48 Justement LB, Campbell KS, Chien NC, Cambier JC : Regulation of B cell antigen receptor signal transduction and phosphorylation by CD45. *Science* 252 : 1839-1842, 1991
- 49 Hurlley TR, Hyman R, Sefton BM : Differential effects of expression of the CD45 tyrosine protein phosphatase on the tyrosine phosphorylation of the lck, fyn, and c-src tyrosine protein kinases. *Mol Cell Biol* 13 : 1651-1656, 1993
- 50 Yanagi S, Sugawara H, Kurosaki M, Sabe H, Yamamura H, Kurosaki T : CD45 modulates phosphorylation of both autophosphorylation and negative regulatory tyrosines of Lyn in B cells. *J Biol Chem* 271 : 30487-30492, 1996
- 51 Ishikawa H, Tsuyama N, Abroun S, Liu S, Li F-J, Taniguchi O, Kawano MM : Requirements of src family kinase activity associated with CD45 for myeloma cell proliferation by interleukin-6. *Blood* 99 : 2172-2178, 2002
- 52 Chin H, Arai A, Wakao H, Kamiyama R, Miyasaka N, Miura O : Lyn physically associates with the erythropoietin and may play a role in activation of the Stat5 pathway. *Blood* 91 : 3734-3745, 1998
- 53 Turkson J, Bowman T, Garcia R, Caldenhoven E, DeGroot RP, Jove R : Stat3 activation by Src induces specific gene regulation and is required for cell transformation. *Mol Cell Biol* 18 : 2545-2552, 1998
- 54 Corey SJ, Dombrosky-Ferlan PM, Zuo S, Krohn E, Donnerberg AD, Zorich P, Romero G, Takata M, Kurosaki T : Requirement of src kinase lyn for induction of DNA synthesis by granulocyte colony-stimulating factor. *J Biol Chem* 273 : 3230-3235, 1998
- 55 Dong F, Lerner AC : Activation of Akt kinase by granulocyte colony-stimulating factor (G-CSF) : evidence for the role of a tyrosine kinase activity distinct from the janus kinase. *Blood* 95 : 1656-1662, 2000
- 56 Liu S, Ishikawa H, Tsuyama N, Li F-J, Abroun S, Otsuyama K-I, Zheng X, Ma Z, Maki Y, Iqbal MS, Obata M, Kawano MM : Increased susceptibility to apoptosis in CD45<sup>+</sup> myeloma cells accompanied by the increased expression of VDAC1. *Oncogene* 25 : 419-429, 2006
- 57 Galbiati F, Razani B, Lisanti MP : Emerging themes in lipid rafts and caveolae. *Cell* 106 : 403-411, 2001
- 58 Janes PW, Ley SC, Magee AI : Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. *J Cell Biol* 147 : 447-461, 1999
- 59 Weintraub BC, Jun JE, Bishop AC, Shokat KM, Thomas ML, Goodnow CC : Entry of B cell receptor into signaling domains is inhibited in tolerant B cells. *J Exp Med* 191 : 1443-1448, 2000
- 60 Sehgal PB, Guo GG, Shah M, Kumar V, Patel K : Cytokine signaling : STATS in plasma membrane rafts. *J Biol Chem* 277 : 12067-12074, 2002
- 61 Melkonian KA, Ostermeyer AG, Chen JZ, Roth MG, Brown DA : Role of lipid modifications in targeting proteins to detergent-

- resistant membrane rafts. *J Biol Chem* 274 : 3910-3917, 1999
- 62 Amano M, Galvan M, He J, Baum LG : The ST6Gal I sialyltransferase selectively modifies N-glycans on CD45 to negatively regulate galectin-1-induced CD45 clustering, phosphatase modulation, and T cell death. *J Biol Chem* 278 : 7469-7475, 2003
- 63 Kawabuchi M, Satomi Y, Takao T, Shimonishi Y, Nada S, Nagai K, Tarakhovskiy A, Okada M : Transmembrane phosphoprotein Csk regulates the activities of Src-family tyrosine kinases. *Nature* 404 : 999-1003, 2000
- 64 Brdicka T, Pavlistova D, Leo A, Bruyns E, Korinek V, Angelisova P, Scherer J, Shevchenko A, Hilgert I, Cerny J, Drbal K, Kuramitsu Y, Kornacker B, Horejsi V, Schraven B : Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase csk and is involved in regulation of T cell activation. *J Exp Med* 191 : 1591-1604, 2000
- 65 Davidson D, Bakinowski M, Thomas ML, Horejsi V, Veillette A : Phosphorylation-dependent regulation of T-cell activation by PAG/Cbp, a lipid raft-associated transmembrane adaptor. *Mol Cell Biol* 23 : 2017-2028, 2003
- 66 Irie-Sasaki J, Sasaki T, Matsumoto W, Opavsky A, Cheng M, Welstead G, Griffiths E, Krawczyk C, Richardson CD, Aitken K, Iscove N, Koretzky G, Johnson P, Liu P, Rothstein DM, Penninger JM : CD45 is a JAK phosphatase and negatively regulates cytokine receptor signaling. *Nature* 409 : 349-354, 2001
- 67 Rajendran L, Masilamani M, Solomon S, Tikkanen R, Stuermer CA, Plattner H, Illges H : Asymmetric localization of flotillins/reggies in preassembled platforms confers inherent polarity to hematopoietic cells. *Proc Natl Acad Sci USA* 100 : 8241-8246, 2003
- 68 Devary Y, Gottlieb RA, Smeal T, Karin M : The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell* 71 : 1081-1091, 1992
- 69 Abe J, Takahashi M, Ishida M, Lee JD, Berk BC : c-Src is required for oxidative stress-mediated activation of big mitogen-activated protein kinase 1. *J Biol Chem* 272 : 20389-20394, 1997
- 70 Abe J, Berk BC : Fyn and JAK2 mediate Ras activation by reactive oxygen species. *J Biol Chem* 274 : 21003-21010, 1999
- 71 Yoshizumi M, Abe J, Haendeler J, Huang Q, Berk BC : Src and Cas mediate JNK activation but not ERK1/2 and p38 kinases by reactive oxygen species. *J Biol Chem* 275 : 11706-11712, 2000
- 72 Abe J, Okuda M, Huang Q, Yoshizumi M, Berk BC : Reactive oxygen species activate p90 ribosomal S6 kinase via Fyn and Ras. *J Biol Chem* 275 : 1739-1748, 2000
- 73 Loeffler M, Kroemer G : The mitochondrion in cell death control : certainties and incognita. *Exp Cell Res* 256 : 19-26, 2000
- 74 Shimizu S, Narita M, Tsujimoto Y : Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. *Nature* 399 : 483-487, 1999
- 75 Shimizu S, Matsuoka Y, Shinohara Y, Yoneda Y, Tsujimoto Y : Essential role of voltage-dependent anion channel in various forms of apoptosis in mammalian cells. *J Cell Biol* 152 : 237-250, 2001
- 76 Takagi-Morishita Y, Yamada N, Sugihara A, Iwasaki T, Tsujimura T, Terada N : Mouse uterine epithelial apoptosis is associated with expression of mitochondrial voltage-dependent anion channels, release of cytochrome C from mitochondria, and the ratio of Bax to Bcl-2 or Bcl-X. *Biol Reprod* 68 : 1178-1184, 2003
- 77 Wang HG, Pathan N, Ethell IM, Krajewski S, Yamaguchi Y, Shibasaki F, McKeon F, Bobo T, Franke TF, Reed JC : Ca<sup>2+</sup>-induced apoptosis through calcineurin dephosphorylation of BAD. *Science* 284 : 339-343, 1999
- 78 Ohtani T, Ishihara K, Atsumi T, Nishida K, Kaneko Y, Miyata T, Itoh S, Narimatsu M, Maeda H, Fukada T, Itoh M, Okano H, Hibi M, Hirano T : Dissection of signaling cascades through gp130 in vivo : Reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. *Immunity* 12 : 95-105, 2000
- 79 Abroun S, Ishikawa H, Tsuyama N, Liu S, Li F-J, Otsuyama K-I, Zheng X, Obata M, Kawano MM : Receptor synergy of interleukin-6 (IL-6) and insulin-like growth factor I in myeloma cells that highly express IL-6 receptor  $\alpha$ . *Blood* 103 : 2291-2298, 2004
- 80 Yamauchi T, Ueki K, Tobe K, Tamemoto H, Sekine N, Wada M, Honjo M, Takahashi M, Takahashi T, Hirai H, Tushima T, Akanuma Y, Fujita T, Komuro I, Yazaki Y, Kadowaki T : Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone. *Nature* 390 : 91-96, 1997
- 81 Podar K, Tai YT, Cole CE, Hideshima T, Sattler M, Hamblin A, Mitsiades N, Schlossman RL, Davies FE, Morgan GJ, Munshi NC, Chauhan D, Anderson KC. Essential role of caveolae in interleukin-6- and insulin-like growth factor I-triggered Akt-1-mediated survival of multiple myeloma cells. *J Biol Chem* 278 : 5794-5801, 2003
- 82 Mitani Y, Takaoka A, Kim SH, Kato Y, Yokochi T, Tanaka N, Taniguchi T : Cross talk of the interferon- $\alpha/\beta$  signalling complex with gp130 for effective interleukin-6 signalling. *Genes Cells* 6 : 631-640, 2001
- 83 Bergsagel PL, Kuehl WM : Chromosome translocations in multiple myeloma. *Oncogene* 20 : 5611-5622, 2001
- 84 Chesi M, Nardini E, Brents LA, Schrock E, Ried T, Kuehl WM, Bergsagel PL : Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat Genet* 16 : 260-264, 1997
- 85 Richelda R, Ronchetti D, Baldini L, Cro L, Viggiano L, Marzella R, Rocchi M, Otsuki T, Lombardi L, Maiolo AT, Neri A : A novel chromosomal translocation t(4;14)(p16.3;q32) in multiple myeloma involves the fibroblast growth-factor receptor 3 gene. *Blood* 90 : 4062-4070, 1997
- 86 Avet-Loiseau H, Li JY, Facon T, Brigaudeau C, Morineau N, Maloisel F, Rapp MJ, Talmant P, Trimoreau F, Jaccard A, Housseau JL, Bataille R : High incidence of translocations t(11;14)(q13;q32) and t(4;14)(p16;q32) in patients with plasma cell malignancies. *Cancer Res* 58 : 5640-5645, 1998

- 87 Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL : The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IGH/MMSET hybrid transcripts. *Blood* 92 : 3025-3034, 1998
- 88 Malgeri U, Baldini L, Perfetti V, Fabris S, Vignarelli MC, Colombo G, Lotti V, Compasso S, Bogni S, Lombardi L, Maiolo AT, Neri A : Detection of t(4;14)(p16.3;q32) chromosomal translocation in multiple myeloma by reverse transcription-polymerase chain reaction analysis of IGH-MMSET fusion transcripts. *Cancer Res* 60 : 4058-4061, 2000
- 89 Iida S, Rao PH, Butler M, Corradini P, Boccadoro M, Klein B, Chaganti RS, Dalla-Favera R : Deregulation of MUM1/IRF4 by chromosomal translocation in multiple myeloma. *Nat Genet* 17 : 226-230, 1997
- 90 Chesi M, Bergsagel PL, Shonukan OO, Martelli ML, Brents LA, Chen T, Schrock E, Reid T, Kuehl WM : Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. *Blood* 91 : 4457-4463, 1998
- 91 Ishikawa H, Tsuyama N, Liu S, Abroun S, Li F-J, Otsuyama K-I, Zheng X, Ma Z, Maki Y, Iqbal MS, Obata M, Kawano MM : Accelerated proliferation of myeloma cells by interleukin-6 co-operating with fibroblast growth factor receptor 3-mediated signals. *Oncogene* 24 : 6328-6332, 2005
- 92 Chung J, Uchida E, Grammer TC, Blenis J : STAT3 serine phosphorylation by ERK-dependent and -independent pathways negatively modulates its tyrosine phosphorylation. *Mol Cell Biol* 17 : 6508-6516, 1997
- 93 Duarte RF, Frank DA : SCF and G-CSF lead to the synergistic induction of proliferation and gene expression through complementary signaling pathways. *Blood* 96 : 3422-3430, 2000
- 94 Ihara S, Nakajima K, Fukada T, Hibi M, Nagata S, Hirano T, Fukui Y : Dual control of neurite outgrowth by STAT3 and MAP kinase in PC12 cells stimulated with interleukin-6. *EMBO J* 16 : 5345-5352, 1997
- 95 Qiu Y, Ravi L, Kung H-J : Requirement of ErbB2 for signalling by interleukin-6 in prostate carcinoma cells. *Nature* 393 : 83-85, 1998
- 96 Nakashima K, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Kawabata M, Miyazono K, Taga T : Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. *Science* 284 : 479-482, 1999