study was approved by the Ethics Committee of Keio University School of Medicine. Ten out of twenty-two evaluable patients (45%) showed more than a 30% reduction of M-protein, and eight (36%) achieved more than a 50% reduction and were associated with restoration of anemia and suppression of normal immunoglobulin level. The clinical course of one of the representative cases is shown in Fig. 2. Somnolence and headache, constipation, peripheral neuropathy and skin rash were frequently observed, but were well tolerated. However, severe granulocytopenia (>50% decrease of pretreatment level) was observed in nine cases. These adverse effects except for granulocytopenia occurred more frequently in patients with a higher plasma concentration of thalidomide (>2.0 µg/ml at twelve hours after the last administration), while granulocytopenia developed regardless of the plasma concentration. Granulocytopenia likely occurred in the patients with reduced function of bone marrow hematopoiesis probably due to previous intensive therapies or by proliferation of myeloma cells in the bone marrow. Recently, deep venous thrombosis has been reported during thalidomide treatment but it was not observed in our study.

We also examined the relationship between the efficacy of thalidomide and the plasma concentrations of angiogenic growth factors before and after treatment. FGF-2 and VEGF levels were frequently elevated before treatment. After thalidomide treatment, VEGF was significantly decreased in responders but not in non-responders. In addition, in two responders, VEGF levels were decreased by the effect of thalidomide, but were increased at recurrence of disease activity. VEGF levels tend to change according to the clinical response. Reduction of angiogenic growth factor levels may be one of the mechanisms for the anti-myeloma effect of thalidomide.

In summary, increased bone marrow MVD as well as plasma concentrations of angiogenic growth factors are correlated with disease activity in MM. These results suggest that bone marrow angiogenesis may be an important factor for disease progression and can also be a therapeutic target. Thalidomide, known as an inhibitor of angiogenesis, was effective for the treatment of refractory MM. However, special caution must be paid to its adverse effects.

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6. Angiogenesis in the Progression of Lung Cancer

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Introduction

Lung cancer is the leading cause of malignancy related

death in Japan since 1998. The high mortality of this disease is associated with the production of distant metastasis to multiple organs by the time of diagnosis. Metastasis consists of multiple events, including the proliferation of tumor cells in the primary site, motility and invasion, transfer by circulation, arrest in the capillary beds in distant organs, adherence to the vessel wall, extravasation, invasion into organ parenchyma, response to microenvironments, and proliferation

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Figure 1. Multiple steps of hematogenous metastasis and requirement of angiogenesis.

(Fig. 1). To supply oxygen and nutrition, angiogenesis is necessary for tumor growth in both primary and distant organs (1). Therefore, angiogenesis is essential for the production of cancer metastasis.

Molecular mechanisms of tumor angiogenesis

Tumor angiogenesis begins with an angiogenic stimulus followed by local degradation of the basement membrane surrounding the capillaries. Endothelial cell migration is then accompanied by the proliferation of cells at the leading edge of the migrating column. As they move, the endothelial cells begin to organize into three-dimensional structures to form new capillary tubes. All of these, then, require endothelial cell survival mandating the interplay of numerous factors, which can act in a positive or negative fashion. The five-step process resulting in neo-angiogenesis is shown in Fig. 2. These events are tightly regulated by various angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietins, interleukin-8 (IL-8), and thymidine phosphorylase (TP). These factors can be produced by not only tumor cells but also host stromal cells (2).

Lung cancer frequently metastasizes to multiple organs, including the bone, lung, brain, and liver. We have

developed several metastasis models and determined molecular mechanisms of multi-organ metastasis of lung cancer (3–7). Brain metastasis is one of the most serious problems, because it restricts the quality of life of the patient and is refractory against various anticancer modalities. We recently reported that tumor-cell derived VEGF induced dilated tumor vessels and facilitated brain metastasis in a nude model (3). Reduction of VEGF expression by antisense-VEGF gene into tumor cells resulted in suppressing the formation of brain metastasis in this model, suggesting that VEGF is an ideal target for prophylaxis of brain metastasis.

Anti-tumor vasculature therapy

There are two strategies against tumor vasculature. The first is the modality targeting angiogenesis; neovascularization during the growth of small tumors (anti-angiogenic agents). The second is the therapy targeting established tumor-associated vessels in large tumors (vascular-targeting agents) (Fig. 3).

As anti-angiogenic agents, a large number of compounds have been developed to inhibit the activity of angiogenic molecules. In particular, neutralizing antibody for VEGF and inhibitors of VEGF receptor tyrosine kinase phosphorylation were evaluated for their therapeutic potential in clinical trials

Figure 2. Molecular mechanism of tumor angiogenesis.

Figure 3. Anti-tumor vasculature therapy.

(Fig. 4). Results of the trials showed that tumor regression was hardly observed when patients with solid tumors in advanced stages were treated with anti-angiogenic inhibitors alone. We also found pre-clinically that a VEGF receptor tyrosine kinase inhibitor (PTK-787) did not significantly inhibit lung metastasis when administered in advanced stages,

even the lung cancer cells expressed high levels of VEGF (8). These findings suggest that single use of anti-angiogenic agents may not be sufficient for controlling tumor progression in the advanced stages. A recent clinical trial showed that the combined use of anti-VEGF antibody with chemotherapy (carboplatin + paclitaxel) significantly improved the

Figure 4. Inhibitors of VEGF signal.

response rate and time to tumor progression in advanced non-small cell lung cancer patients (9). Chemotherapy reduces the tumor volume and anti-VEGF antibody may inhibit the re-growth of tumors by suppression of the angiogenesis. Therefore, the combined use of anti-angiogenic agents with other modalities may be useful for lung cancer.

Vascular targeting agents that damage established tumor vasculature are also of potential clinical use. Tubulin inhibitors (combretastatin A4 phosphate, ZD6126) disrupt tubulin dimerization, cause damage to endothelial cells, and shut off blood flow. In one case, the anaplastic thyroid tumor completely disappeared after treatment with combretastatin (10). We have shown that another vascular targeting agent, ZD6126, shut down blood flow, inducing tumor necrosis within 24 hours (11). Importantly, ZD6126 induces apoptosis of endothelial cells in tumors, but not in non-tumor parenchyma, indicating the selectivity of this drug to tumor vasculature. The therapeutic efficacy of these drugs is being evaluated in clinical trials.

Epidermal growth factor receptor (EGF-R) is one of the molecular targets for solid tumors, including non-small cell

Figure 5. Rational of molecular target drugs in combination with established anticancer modalities.

lung cancer. EGF-R is overexpressed on the cell surface of various types of tumors. Signals via EGF-R directly stimulate cell proliferation, prevent apoptosis, indirectly facilitate production of angiogenic cytokines, and hence promote tumor progression. Recently, EGF-R inhibitors were shown to have therapeutic potential against chemotherapy-refractory non-small cell lung cancer.

Conclusion

Understanding the molecular pathogenesis in the progression of lung cancer may facilitate the development of new therapeutic modalities by which critical molecules involved in malignant phenotypes of lung cancer can be therapeutically targeted. These molecular targeted therapeutics, including anti-tumor vasculature agents and EGF-R inhibitors, seem to be generally less-toxic and/or cytostatic against cancer. This approach may provide a new strategy for the therapy of lung cancer in combination with conventional anti-cancer therapy (surgery, radiation therapy, chemotherapy) (Fig. 5) in terms of survival prolongation and improvement of QOL in lung cancer patients.

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