General Summary of Division of Tumor Dynamics and Regulation

Growth factors and their receptors of tyrosine kinase play fundamental roles in diverse processes, including development, morphogenesis, cell growth, and tissue regeneration. Because of this, aberrant regulation of receptor tyrosine kinases participates in the development and progression of a variety of human cancers. Among malignant behavior of tumors, tumor metastasis is the most important factor affecting survival of cancer patients. Approximately 90% of all cancer death arise from the metastatic spread of primary tumors. Although mutational alterations and epigenetic events that occur in oncogenes and tumor suppressor genes are of a genetical background for tumorigenesis, invasive and metastatic behavior of tumor cells is largely regulated by extracellularly acting growth factors. Among them, hepatocyte growth factor (HGF) particularly affects metastatic behavior in a wide variety of cancer cells.

The signal exchange, cell-cell communications, and the genetic programs that function during tissue regeneration are evoked in cancer tissues. Tumors are likely to be wounds that do not heal (Dvorak, 1986). In normal tissues, HGF-Met signaling plays diverse roles in organogenesis and healing of wounded tissues, but aberrant regulation in HGF-Met signaling confers highly invasive and metastatic characteristic in a variety of cancer cells. Despite the appreciation of the clinical relevance of tumor metastasis, therapeutic intervention that can efficiently prevent metastasis has yet appeared. If metastatic tumors can be suppressed to non-metastatic tumors, the rate of cancer cures is considered to show much improvement. Inhibition of HGF-Met signaling has been considered to provide ways to prevent cancer metastasis.

We discovered NK4 as a competitive inhibitor against HGF-Met. NK4 is a fragment of HGF, consisting of an N-terminal hairpin domain and four kringle domains. NK4 binds to the Met but does not activate Met, thereby competitively inhibiting HGF-dependent activation of Met. We thereafter found that NK4 functions as an angiogenesis inhibitor, and that this angiostatic activity of NK4 is probably independent of its original activity as an HGF-antagonist. Based on unique biological actions of NK4, anti-cancer effect of NK4 has been demonstrated in different types of malignant tumors in mice. We recently addressed therapeutic effect of NK4 on malignant tumors such as colon cancer and malignant pleural mesothelioma. On the other hand, based on advances in bioinformatics and protein crystallography, new approaches to discover small molecule inhibitors for specific target proteins have progressed. Application of such new drug discovery technologies is exciting to discover molecules targeting HGF-Met.

HGF and its receptor Met tyrosine kinase play roles particularly in dynamic morphogenesis, cellular locomotion, and epithelial-mesenchymal interaction/transition during development and

tissue regeneration. In the adult, HGF-Met signaling is activated in response to tissue injuries for regeneration of various organs, including the liver. Administration of HGF suppresses the onset of pathology, facilitates tissue regeneration, and improves pathology such as tissue fibrosis. For the clinical development of HGF as regenerative medicine, clinical trials of HGF for treatment of skin ulcer and acute renal failure started in 2009.

During basic research to know mechanisms for tissue regeneration by HGF, we found that HGF exerts biological activity in "the injured tissue-selective manner," even when HGF is administered systemically. The injured tissue-selective activation of the Met receptor suggests that in the injured tissue Met is activated upon stimulation with HGF, but in non-injured (normal) tissues and the regenerated tissue after wounding, Met receptor activation is suppressed even after stimulation with HGF. Understanding of the mechanism by which Met activation is regulated in response to tissue injury or intactness may be a clue to know the fundamental question on how the mass and organization of tissues are maintained before and after regeneration. Likewise, loss-of-function in the negative regulation of Met activation is considerable as a possible mechanism for an aberrant activation of Met in cancer.

A) Anti-cancer Approach with NK4 and Anti-angiogenic Mechanism of NK4

The mechanism by which NK4 inhibits angiogenesis remained unknown. We recently found that NK4 inhibited extracellular assembly of fibronectin, by which integrin-dependent anchoring signal transduction was inhibited by NK4 in endothelial cells. Based on unique bifunctional characteristic of NK4, therapeutic approaches with NK4 have been examined in experimental models, including colon cancer, pleural malignant mesothelioma, multiple myeloma, and hepatoma. In these models, NK4 protein administration or NK4 gene therapy inhibited tumor invasion and metastasis, and angiogenesis-dependent tumor growth.

B) Suppression of Met/HGF Receptor Activation by the Met Juxtamembrane function and Cell-Cell Contact

We previously showed that Met activation is suppressed by Ser985 phosphorylation in the juxtamembrane (JM) domain of Met. Since the Met deleted with the JM domain naturally exists as a splicing variant, the JM Ser985 phosphorylation may play an important physiological role. On the other hand, because the contact inhibition is fundamental characteristic of normal cells, we hypothesized that Met activation might be regulated by cell-cell contact. We found the suppressive mechanism for Met activation by cell-cell contact, using hepatocytes in primary culture. The cell-cell contact up-regulated expression of LAR protein tyrosine phosphatase and LAR dephosphorylated Met, by which Met activation was suppressed even after stimulation with HGF. Therefore, we found two different mechanisms involved in suppression of Met activation even when

cells are stimulated with HGF. The one is cell-cell contact-dependent inactivation of the Met by LAR. The other is suppression of Met activation by the JM Ser985 phosphorylation.

C) In Silico-Based Drug Discovery Targeting HGF-Met

HGF-Met pathway is a hot target in worldwide discovery of molecular target therapy of cancer. To discover new small molecule inhibitors for HGF-Met pathway, a research group (K. Matsumoto as a head researcher) was organized by researchers of different specialties (bioinformatics, protein chemistry, protein crystallography). In this research, small molecule inhibitors as lead compounds were discovered by successive in silico-based drug discovery techniques. Analysis of the crystal structure of the complex composed of a target protein and a compound is in progress for potentiation and optimization of lead compounds.