Meeting Report

Transforming growth factor-β signaling and cancer: The 28th Sapporo Cancer Seminar, 25–27 June 2008

Kohei Miyazono

Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113–0033, Japan

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The Sapporo Cancer Seminars have been held annually since 1981. The 28th Sapporo Cancer Seminar was held on 26–27 June 2008 at the Hokkaido University Conference Hall, focusing on transforming growth factor (TGF)-β signaling and cancer. More than 150 scientists participated in the seminar, and it provided a great deal of information on the role of TGF-β signaling in carcinogenesis and tumor metastasis. The possible use of TGF-β antagonists for treatment of cancers was also discussed at the seminar. (*Cancer Sci* 2009; 100: 363–365)

Transforming growth factor (TGF)-β was discovered nearly 30 years ago, when it was found to induce reversible transformation of normal fibroblasts in soft agar. Following its discovery, TGF-β was shown to be a pleiotropic cytokine, which regulates various cellular functions including cell proliferation, differentiation, apoptosis, and migration. TGF-β is a potent inhibitor of the growth of most types of cells, including epithelial cells, endothelial cells, and lymphocytes. TGF-β also induces deposition of extracellular matrix proteins, and thus induces tissue fibrosis. TGF-β binds to type I and II serine-threonine kinase receptors, known as TβRI and TβRII, respectively, and activates intracellular signaling molecules, including Smad family proteins. TGF-β phosphorylates Smad2 and Smad3, which form complexes with Smad4 and regulate the transcription of target genes after moving into the nucleus. In addition to the canonical Smad pathway, recent findings have shown that TGF-β activates non-Smad signaling pathways that play important roles in the regulation of development and carcinogenesis.

TGF-β has bidirectional effects on the progression of cancers. It acts as an anti-oncogenic factor by inhibiting cell proliferation in the early phases of carcinogenesis, whereas in the late phases of carcinogenesis it functions as a pro-oncogenic factor by stimulating the epithelial–mesenchymal transition and by regulating immune function and tumor angiogenesis. Recently, antagonists that regulate the activity of TGF-β have been developed. These TGF-β antagonists may be useful for the treatment of some fibrotic diseases induced by TGF-β, as well as for the treatment of certain cancers, especially in the prevention of cancer metastasis. Because of accumulating evidence that TGF-β plays complex roles in the progression of cancer and the discovery of potent TGF-β antagonists, we considered it a very good time to organize an international meeting and discuss recent advances concerning TGF-β signaling and its roles in cancer.

At the 28th Sapporo Cancer Seminar, we discussed: (i) molecular mechanisms of TGF-β signaling; (ii) TGF-β signaling and carcinogenesis; (iii) TGF-β signaling and cancer metastasis; and (iv) development and application of TGF-β-based therapeutic agents. We invited outstanding researchers from the USA, Sweden, the Netherlands, China, and Japan. In addition, some poster presentations were selected for oral presentation to facilitate discussion. More than 150 scientists from Japan, China, Korea, the USA, Sweden, the Netherlands, and Hungary, participated in the seminar (Fig. 1). Many unpublished data were presented at the seminar, and we had a wonderful time exchanging findings.

Molecular mechanisms of TGF-β signaling

After an introduction and overview of the field by Kohei Miyazono (Tokyo, Japan) on 26 June, Ye-Guang Chen (Beijing, China), Mitsuyasu Kato (Tsukuba, Japan), Rik Derynck (San Francisco, CA, USA), and Daizo Koinuma (Tokyo, Japan) discussed mechanisms of TGF-β signaling, including novel regulators of the TGF-β signaling pathway and mechanisms of transcriptional regulation of TGF-β signaling.

When normal cells transit to malignant cells, they often exhibit altered responses to TGF-β with a decrease in the anti-proliferative effects of TGF-β. Some of these alterations result from irreversible mutations of certain oncogenes and anti-oncogenes, whereas other changes in TGF-β responsiveness result from more dynamic and reversible changes in signaling mechanisms. One important step in regulation of the TGF-β response may occur through post-translational modifications of the TβRI protein. The protein level of available TβRI has been reported to be controlled by ubiquitylation. Rik Derynck presented new findings suggesting that the activity of TβRI is regulated by sumoylation.

Sumoylation occurs in various intracellular proteins, but has been primarily characterized as a functional modification of nuclear proteins, such as transcription factors. Rik Derynck found that TβRI is sumoylated in response to TGF-β, and that this sumoylation requires the kinase activities of both TβRI and TβRII. Sumoylation of TβRI enhances receptor function by facilitating the recruitment and phosphorylation of Smad3, leading to regulation of TGF-β-induced transcription and growth inhibition. Interestingly, he showed that sumoylation occurs only on TβRI and not other type I receptors, and it modulates metastasis of transformed cells in a mouse model. Sumoylation of TβRI thus controls the magnitude of the response of cells to TGF-β. TβRI is the first cell surface receptor whose function has been shown to be modified by sumoylation.

Luncheon seminar

At the luncheon seminar, Erik Nystrom (Uppsala, Sweden) introduced a novel technology for visualization of protein–protein interactions, called the '*in situ* proximity ligation assay'. Using this technique, Katerina Pardali (Uppsala, Sweden) monitored the dynamics of formation and dissolution of complexes of

[1]To whom correspondence should be addressed. E-mail: miyazono-ind@umin.ac.jp

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Fig. 1. Participants of the 28th Sapporo Cancer Seminar.

endogenous Smad proteins during TGF-β signaling. This interesting method may allow monitoring of the status of activation of the TGF-β signaling pathway in patient tissue samples as well as detection of TGF-β family signaling in development. In addition, it may be used for high-throughput screening of compounds designed to block the formation of Smad complexes.

TGF-β signaling and carcinogenesis

In the afternoon session of 26 June, Carl-Henrik Heldin (Uppsala, Sweden), Kunxin Luo (Berkeley, CA, USA), Shirin Bonni (Calgary, AB, Canada), Katja Bruckner (San Francisco, CA, USA), and Youn Sook Lee (SungKyunKwan, Korea) discussed TGF-β signaling and its roles in carcinogenesis, focusing in particular on various molecules that regulate TGF-β signaling. Angiogenesis plays an important role in the development of cancer and metastasis. Peter ten Dijke (Leiden, the Netherlands), and Fumiko Itoh (Tsukuba, Japan) discussed the roles of TGF-β and its receptors in angiogenesis.

TGF-β-associated kinase (TAK) 1 is a member of the mitogen-activated protein (MAP) kinase kinase kinase family, which was originally identified as an effector of TGF-β-induced activation of p38 MAP kinase. Carl-Henrik Heldin reported that the E3 ubiquitin ligase tumor necrosis factor receptor-associated factor 6 (TRAF6) binds to a consensus motif present in TβRI. The interaction between TβRI and TRAF6 is required for auto-ubiquitylation of TRAF6 induced by TGF-β and subsequent activation of the TAK1–p38–c-Jun N-terminal kinase (JNK) pathway, which leads to apoptosis of cells. Interestingly, the kinase activity of TβRI is not required for the activation of TAK1 by TRAF6. He concluded that, in addition to the canonical Smad pathway, TGF-β activates TAK1 via interaction of TβRI with TRAF6, and that TAK1 then activates downstream p38 and JNK MAP kinase pathways.

TGF-β signaling and cancer metastasis

In the morning session of 27 June, Mark Taketo (Kyoto, Japan), Xiao-Fan Wang (Durham, NC, USA), Theresa Guise (Charlottesville, VA, USA), Thomas Doetschman (Tucson, AZ, USA), Koichi Matsuzaki (Osaka, Japan), and Motoko Shibanuma (Tokyo, Japan) discussed TGF-β signaling and invasion and metastasis of cancer. Some animal models were introduced by Mark Taketo, Theresa Guise, and Thomas Doetschman.

Most colorectal adenomas are initiated by inactivation of the adenomatous polyposis coli (APC) gene, and progress to malignant adenocarcinoma through additional mutations in some oncogenes and anti-oncogenes. Mark Taketo had previously generated a compound mutant mouse strain *cis-Apc*$^{+/\Delta 716}$*Smad4*$^{+/-}$ (*cis-Apc/Smad4*), in which intestinal adenomas transform to invasive adenocarcinomas. He has now demonstrated that a novel type of immature myeloid cell (iMC) is recruited from the bone marrow to the tumor invasion front in *cis-Apc/Smad4* mice. These iMC are CD34-positive, express matrix metalloproteinase (MMP) 9, MMP2, and CC-chemokine receptor (CCR) 1, and migrate toward the CCR1 ligand CCL9. In the adenocarcinomas, levels of CCL9 expression were increased in the tumor epithelium. He showed that loss of CCR1 in the *cis-Apc/Smad4* mutant mice prevented the accumulation of CD34^{+} iMC at the invasion front and suppressed invasion of tumors. His findings thus demonstrate therapeutic implications of using inhibitors of the CCL9–CCR1 axis in treating invasive colon cancer.

Development and application of TGF-β-based therapeutic agents

In the afternoon session of 27 June, Takeshi Imamura (Tokyo, Japan), Lalage Wakefield (Bethesda, MD, USA), Mitsunobu Kano

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(Tokyo, Japan), and Hideaki Ijichi (Tokyo, Japan) presented their findings regarding *in vivo* imaging and possible use of TGF-β inhibitors for the treatment of cancers.

TGF-β antagonists have exhibited efficacy against tumors in pre-clinical models with few of the predicted toxicities. Lalage Wakefield and her colleagues have carried out detailed mechanistic analyses of the effects of an anti-TGF-β monoclonal antibody using the 4T1 mouse model of metastatic mammary cancer. She showed that the TGF-β antibody enhanced various immune activities involving both innate and adaptive immunity, and has therefore referred to this process as 'death by a thousand cuts'. Effects of the anti-TGF-β antibody included increased numbers of infiltrating natural killer cells, expression of the coactivating receptor NKG2D on infiltrating $CD8^+$ T cells, and enhanced susceptibility of tumor cells to immune cell-mediated recognition and cell lysis. The anti-TGF-β antibody also prevented tumor cells from subverting $CD8^+$ T cells into a form producing interleukin-17 and supporting tumor cell survival. These individually small effects function to activate antitumor immune responses, and prevent the process of tumorigenesis and metastasis without autoimmune complications.

Conclusion

The present international symposium was quite successful, with many excellent talks and much fruitful discussion. The signaling by TGF-β and its family molecules has a variety of functions and plays important roles in many diseases, especially cancer. The functions of its signaling system in the genesis and progression of cancer have been reported to be complex and of profound importance. In the present conference, we discussed a wide spectrum of functions of TGF-β family signaling in cancer, including potential applications of signaling to therapeutics, with numerous world-class investigators. We were very pleased that researchers from many countries participated in this seminar and discussed their most recent findings. The seminar was held in a friendly atmosphere. We hope that many collaborative projects will evolve from the personal contacts established during the seminar. Finally, we would like to acknowledge the generous support of the Sapporo Cancer Seminar Foundation, which gave us the opportunity to organize this exciting seminar. We believe that all participants had fruitful discussions during this seminar, and enjoyed their stay in the beautiful city of Sapporo.

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