Influence of a Master

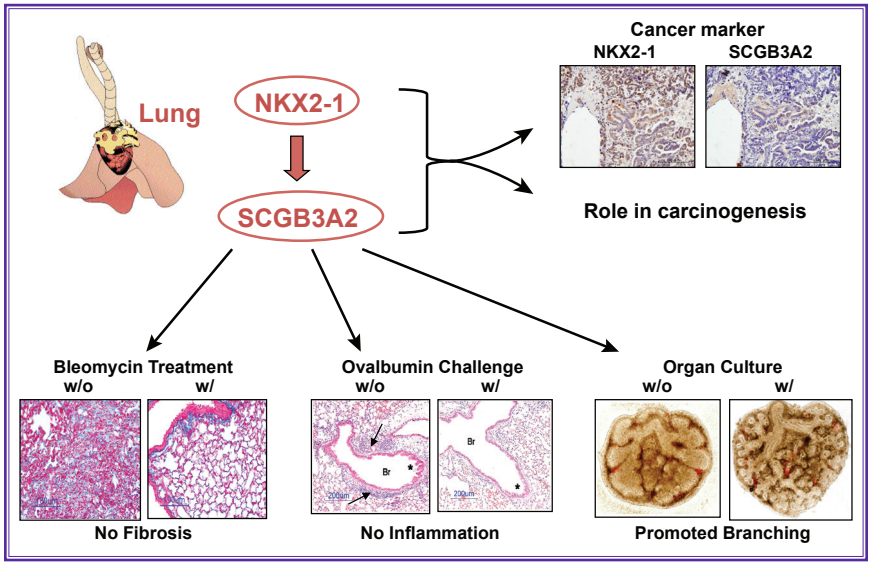
In 1991, Shioko Kimura, Ph.D., Senior Investigator in CCR's Laboratory of Metabolism, cloned the transcription factor Thyroid-specific enhancer binding protein (T/EBP) based on its ability to bind to an enhancer region in the promoter of the thyroid peroxidase gene. NKX2-1, as the 38-kDa protein is now known, plays a central role in early development of the lung, thyroid, and ventral forebrain; its expression is also associated with cancers of the lung and thyroid. Kimura's laboratory has made several genetically engineered mouse models that she has shared with the research community, while continuing to focus her interests on development and cancer of the thyroid and lung. Her laboratory has discovered a molecule downstream of NKX2-1, secretoglobin (SCGB) 3A2, on which they have recently filed patents for its therapeutic potential.

Master Regulator

"Years ago, I was working on the regulation of thyroid peroxidase gene expression," began Kimura. Thyroid peroxidase is an important enzyme in the production of thyroid hormones from thyroglobulin. "I cloned the thyroid peroxidase gene and was analyzing the promoter, which led me to a protein binding 5.5 kB upstream of the transcription start site." This protein, which she called T/EBP, was also found by a group in Italy and named thyroid transcription factor-1 (TTF-1), a transcriptional activator of the thyroglobulin gene which binds a homeodomain with strong homology to the *Drosophila* NK2 homeodomain. NKX2-1, as the transcription factor is now known, is a master regulator of many more thyroid and lung-specific genes, and the proteins encoded by these genes are essential for the function and homeostasis of the respective organs.

Kimura's work on NKX2-1 as a developmental transcription factor led her to its role in cancer. Her laboratory has created several mouse models to study NKX2-1 function, including mice that express *Nkx2-1* in the thyroid at half the level of the normal mouse thyroid. However, the expression varies at the individual cell level (as distinct from heterozygous mice that have *Nkx2-1* expression reduced by half in all cells within the tissue). Approximately 20 percent of these mice exhibited atrophic/degenerative thyroids and frequent adenomas, while the rest developed thyroids with extraordinarily dilated follicles, suggesting a role for NKX2-1 in disease. Based on these results, her team decided to test the susceptibility of these mice to cancer induced by a genotoxic carcinogen. They found an increased incidence of thyroid adenomas associated with a doubling of the normal cell proliferation rate. Human thyroid cancer association studies have also found a link with the human homologue *NKX2-1.*

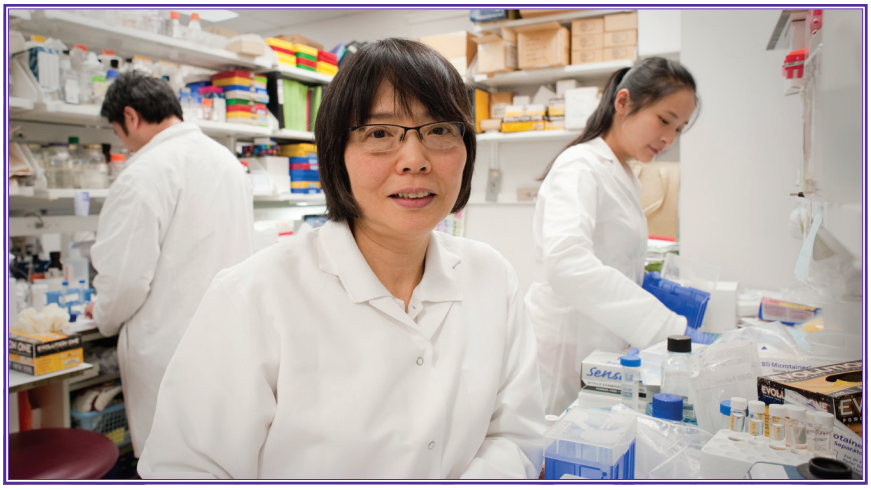
Kimura has followed the trail of NKX2-1 as it relates to thyroid development and carcinogenesis, while collaborating with other laboratories to explore its roles in the lung. "We are also interested in the forebrain, and have shared our mice with collaborators who specialize in brain development and function, but so far this line of investigation has



(Figure: S. Kimura, CCR)

SCGB3A2 was identified as a downstream target for NKX2-1, one of the master transcription factors in lung. SCGB3A2 demonstrated 1) anti-fibrotic activity in the bleomycin-induced pulmonary fibrosis model mouse, 2) anti-inflammatory activity in the ovalbumin allergic inflammation model mouse, and 3) growth factor activity promoting lung development as shown by *ex vivo* embryonic lung organ culture. SCGB3A2 can be used as a tumor marker just like NKX2-1, which has a role in lung carcinogenesis and is an established diagnostic marker for lung adenocarcinomas. The role of SCGB3A2 in lung carcinogenesis is unfolding.

(Photo: R. Baer)



Shioko Kimura, Ph.D., (center) with Shigetoshi Yokoyama, Ph.D., and Yan Cai, M.D., Ph.D., working in the lab

been slow due to the complexity of working with this organ."

Most recently, she has shared her expertise and transgenic mice with the laboratory of Tyler Jacks, Ph.D., at the Massachusetts Institute of Technology; and, they recently co-authored an analysis of conditional *Nkx2-1* disruption in normal and cancerous lungs. NKX2-1 expression is seen in 75 to 85 percent of human lung adenocarcinomas, where it is associated with a better prognosis. Their work suggests NKX2-1 is important for maintaining a pulmonary differentiation state that results in a less aggressive cancer.

Downstream Hope

In 2001, Kimura and her colleagues identified a novel target gene of NKX2-1, Uteroglobin/Clara Cell Secretory Protein-Related Protein (UGRP1), which is primarily expressed in lung airway epithelial cells, but also found in the thyroid. It was later recognized as part of the secretoglobin (*Scgb*) gene superfamily, which is involved in lung inflammation; it was thus renamed SCGB3A2.

SCGB3A2 is a secretory protein, whose receptor is unknown. Like the most studied founding member of the *Scgb* gene superfamily, SCGB1A1, it has significant anti-inflammatory activity. Kimura's laboratory has identified roles for the protein as a growth factor required for fetal lung development. It also has antifibrotic activity.

In 2007, based on her work on this molecule, Kimura and NCI filed a patent to use SCGB3A2 or a molecular mimic to treat the development of neonatal respiratory distress, promote lung development, and reduce lung damage due to fibrosis that results from certain anticancer agents. She is working with a local biotech company to advance this agent to the clinic.

"When we found that this protein has anti-inflammatory activity combined with growth-promoting activities, I felt there should be a connection to cancer," said Kimura. She and her colleagues went on to demonstrate that SCGB3A2 is overexpressed in lung carcinomas, particularly in human adenocarcinomas. More recently, they discovered that SCGB3A2 may also have anticancer activity and have broadened their intellectual property accordingly. They have shown in more than one mouse model, that administration of SCGB3A2 reduces tumor burden and metastatic migration to the lung.

"We are working hard to clarify the secretoglobin signaling pathway and how its antitumor activity works; we want to understand how widely this can be applied to adult cancers and whether, if we can target this protein, we can decrease lung cancer incidence and metastasis," said Kimura. And of course, if they can identify SCGB3A2's receptor, they will address many more questions about its function.

"We have been working for 7 or 8 years to find the receptor for this molecule. We've done everything: cDNAs, overexpressions, pull downs… Receptor isolation and identification is just not that easy to do," said Kimura. "But recently, we've found a candidate through protein-protein interaction arrays which is a potential receptor in tumors and normal tissues. Finally, we see a light at the end of the tunnel."

Under Construction

Kimura's laboratory is also working on establishing cell lines from adult thyroid stem cells, a challenge all the more daunting because there are no known thyroid stem/progenitor cell-specific surface markers. "The thyroid is so small in mice that for one study, you need 30 to 40 mouse thyroids and then you can only do one or two experiments," said Kimura. "We are establishing a cell line to see if we can produce a more stable source of thyroid stem cells."

Once established, the cell line could be manipulated to address several research questions. It could be transformed as a model of cancer initiation. Kimura also suspects that NKX2-1 may be involved in stem cell niche maintenance, a hypothesis she plans to test once she can establish the cells.

As with her work on the thyroid peroxidase gene, which started her on the trail of NKX2-1, Kimura knows that the true value of an experimental pursuit is as much in the new questions it enables as in the answers it provides.

To learn more about Dr. Kimura's research, please visit her CCR website at http://ccr.cancer.gov/staff/staff.asp?name=kimura.