

Abstracts of the 2010th Maruyama Memorial Lectures of the 79th Annual Meeting of the Medical Association of Nippon Medical School

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Abstracts of the 2010th Maruyama Memorial Research Fund Prize Memorial Lecture (I)

Effectiveness of Nestin-targeting Therapy in Pancreatic Cancer

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Nestin, a class VI intermediate filament protein, has been reported to be a progenitor marker in various tissues¹, including a tumor vessel marker in colorectal cancer² and prostate cancer and an exocrine progenitor cell marker in the pancreas. We have previously reported that nestin expression is detected in approximately 30% of cases of pancreatic cancer and is associated with tumor invasion³. Therefore, we hypothesized that nestin-targeting therapy, by modulating both cancer cell invasion and tumor angiogenesis, would be an effective treatment for pancreatic cancer.

To clarify the functional roles of nestin, we constructed nestin-short hairpin (sh) RNA-transfected pancreatic cancer cells. Knockdown of nestin in pancreatic cancer cells inhibited migration, invasion, and metastasis *in vivo*⁴. Furthermore, decreased nestin expression inhibited sphere forming ability, which is a cancer stem cell characteristic. Alterations in the expression levels of genes involved in tumor metastasis were analyzed in nestin shRNA-transfected pancreatic cancer cells using a polymerase chain reaction array (SABiosciences Corporation, Frederick, MD, USA). Gene networks and pathways representing key genes were identified with the Ingenuity Pathways Analysis (IPA) database (Ingenuity Systems, Inc., Redwood, CA, USA) (**Fig. 1**). With the IPA database, networks were generated as graphical representations of the molecular relationships between genes and gene products. The colors of genes in the networks indicate up-regulation (red) or down-regulation (green). The data revealed that decreased nestin expression induced alterations in the expression of molecules important in cancer development, including TP53, RB, TGFB, MYC, and KRAS. These findings support previous reports that nestin is involved in cytoskeletal organization and directly binds several proteins, such as cyclin-dependent kinase 5⁵ and glucocorticoid receptor⁶, and thus regulates the cell cycle, cell division, differentiation, and cell organization.

Next, we investigated whether knockdown of nestin had antitumorigenic activity by using short interfering (si) RNA targeting nestin *in vitro* and *in vivo*. *In vitro*, nestin siRNA inhibited the growth of mouse vascular endothelial cells. For *in vivo* experiments, were human pancreatic cancer cells subcutaneously implanted in nude mice, after which intratumor injections of siRNA targeting mouse nestin were performed. This knockdown of nestin decreased both tumor volume and tumor vessel density.

In conclusion, our data indicate that nestin is an important regulator of tumor angiogenesis and of the

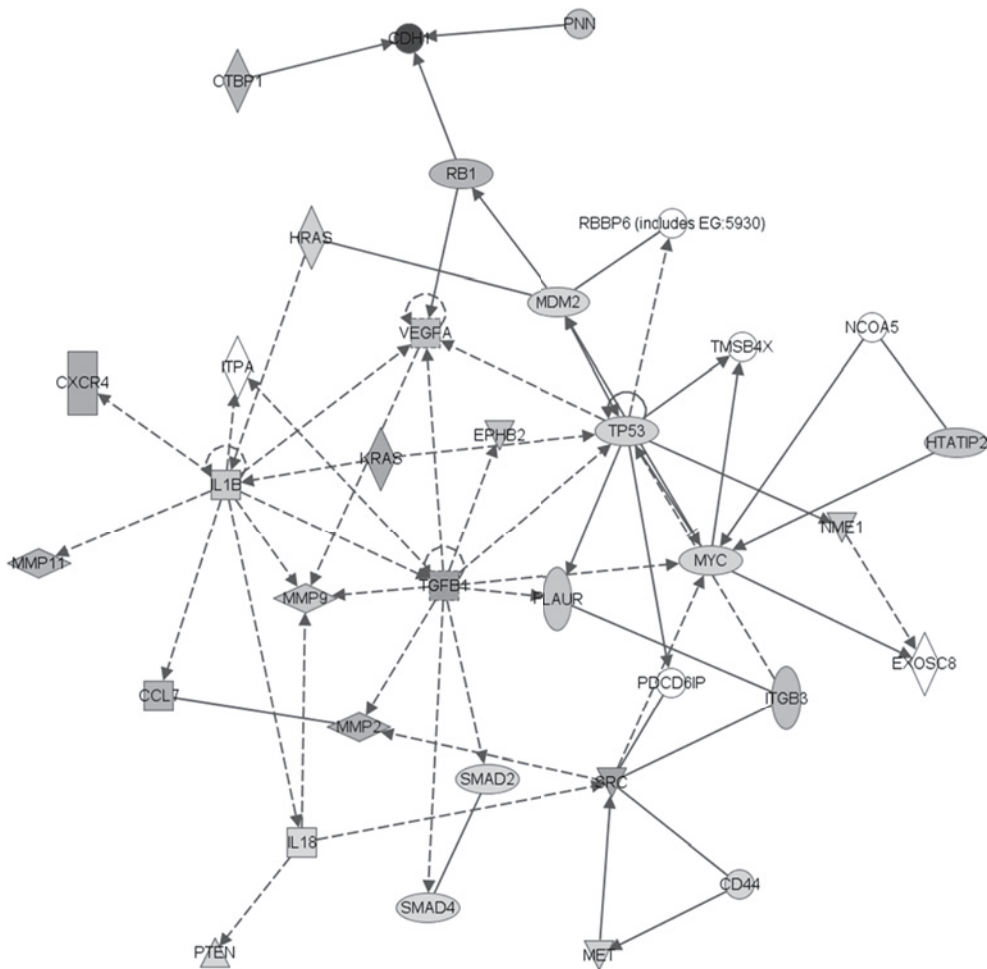


Fig. 1 Network diagram generated as a graphical representation of the molecular relationships between genes and products
 The gene products are represented as nodes (shapes) and the biological relationship between two nodes is represented as an edge (line). All edges are supported by at least one reference stored in the Ingenuity Pathways Knowledge Base. Solid line, direct interaction; dotted line, indirect interaction; arrowed line, acts on; straight line, binding only.

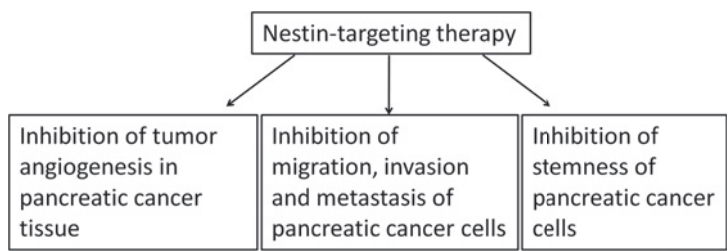


Fig. 2 Nestin-targeting therapy in pancreatic cancer
 Nestin is a promising novel candidate for pancreatic cancer therapy via inhibition of cell migration, invasion, metastasis, stemness, and tumor angiogenesis.

aggressiveness of pancreatic cancer (**Fig. 2**). Nestin plays important roles in the functions of pancreatic cancer stem cells and thus represents a novel candidate for pancreatic cancer therapy.

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