Novel Functions of HST-1/FGF-4

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Abstract: The HST-1 gene, originally identified in our laboratory as a transforming gene, encodes HST-1 protein or fibroblast growth factor (FGF)-4, a member of the FGF family. In this study, to explore a novel function of HST-1 gene, gene knock-in (gain of function) and knock-out (loss of function) were performed.

To identify the target cells and tissues which are susceptible to the biological actions of HST-1 protein *in vivo*, we gave mice a recombinant adenovirus carrying the *HST-1* gene (AdexHST-1). The results show that AdexHST-1 caused a two-fold increase in peripheral platelet count for a period of 30 days, and also effectively prevented experimentally induced thrombocytopenia. We further elucidated that thrombopoietic action of HST-1 is achieved in that HST-1 can regulate megakaryocyte development as a megakaryocyte potentiator synergistically with IL-3 and TPO as well as potentiates megakaryocyte maturation.

As a means of exploring the role of HST-1 in vertebrate embryogenesis, mice with disrupted *HST-1* gene were produced by homologous recombination. Null mutant ES cells survived but homozygous HST-1^{-/-} mutants showed severe developmental delay and died before 9 days of gestation, suggesting that HST-1 plays essential roles in early phases of mouse development.

In addition to its early embryonic function, HST-1 may play a crucial role in the later stage of development. In fact, we and others have found that HST-1 is expressed in the cells of the apical ectodermal ridge of mouse limb bud at 11 and 12 days of gestation. In an effort to further understand the role of HST-1 in mouse limb development, we initially established a novel limb culture system which provides us with a suitable model system to explore the key factors for limb development. Using this culture system, we have shown that exposure of embryonal limb bud explants to antisense oligonucleotides of HST-1 blocks limb outgrowth. Our results suggest direct evidence that HST-1 worked naturally as an inducer of mouse limb development. (J Toxicol Pathol 1997; 10: 129~132)

Key words: HST-1/FGF-4, Adenovirus vector, Platelet, Knockout, ES cells, Limb, Antisense

Introduction

In mammals, there are presently at least nine genes that have been classified as family members of the fibroblast growth factors (FGFs)¹⁻⁴. FGFs are mitogenic to a large number of ectodermally and mesodermally derived cells and can act as inducers of cell differentiation⁵⁻⁷. The *HST-1* (FGF-4) gene was first identified as a transforming gene from human stomach cancer⁸, and encodes FGF-4, a 206-amino acid protein. Unlike the two prototypes of the FGF gene family, basic FGF and acidic FGF, HST-1 contains an intact signal peptide, characteristic of secreted proteins, and is glycosylated and secreted in the medium of producer cells. Like other members of the FGF family, HST-1 possesses heparin-binding sites, and stability of the secreted protein is enhanced by heparin. Its transcript was identified in male germ cell tumors⁹, embryonic stem (ES) cells, embryonal carcinoma (EC) cells¹⁰, and several other solid malignant tumors including Kaposi's sarcoma and melanoma. The human HST-1 gene is located at chromosome 11q13, and frequently amplified in a variety of human tumors¹¹. However, the gene is normally silent in normal adult tissues, giving rise to the presumption that HST-1 protein (FGF-4) is important during embryonic development. In EC cells,

enhancer analysis showed that developmental-specific activity of the HST-1 enhancer requires the synergistic action of Sox2 and Oct-3.

In this study, to explore a novel function of HST-1 gene *in vivo*, studies of gene knock-in (gain of function) and knock-out (loss of function) were performed.

HST-1 acts as a megakaryocyte potentiator *in vivo*

In order to know the biological function of HST-1 *in vivo*, we injected a replication-deficient adenovirus containing the *HST-1* gene (AdexHST-1) into normal mice systemically. AdexHST-1 caused a two-fold increase in peripheral platelet count in animals¹². We were able to show that the HST-1 product works effectively in many steps in megakaryocyte maturation and elucidated that HST-1 acts as a megakaryocyte potentiating factor. We also demonstrated that a single intraperitoneal injection of AdexHST-1 into mice quite effectively prevented platelet count decrease induced by administration of a chemotherapeutic agent or by irradiation¹³. Administration of AdexHST-1 caused a continuous HST-1 protein expression and increased the count of large megakaryocytes in bone marrow, which caused platelet count recovery and diminished the extent and duration of thrombocytopenia, with no detectable side effects. Therefore, our method can be useful for treatment and/or prevention of thrombocytopenia (Fig. 1).

Various growth factors and cytokines affecting megakaryocytopoiesis and thrombocytopoiesis *in vitro* and *in vivo* were reported such as interleukin-1 (IL-1), IL-3, IL-6, IL-

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Fig. 1. Effective prevention of thrombocytopenia by AdexHST-1 : HST-1 acts as a megakaryocyte potentiator (Meg-POT) on megakaryocyte. Adenovirus vector carrying HST-1 gene will be useful for the prevention and treatment of thrombocytopenia induced by chemotherapy or X-ray irradiation in cancer treatment.

11, c-kit ligand (stem cell factor), erythropoietin, granulocyte-macrophage colony-stimulating factor (GM-CSF), aFGF, bFGF, and thrombopoietin (TPO)^{14,15}. We further elucidated that thrombopoietic action of HST-1 is achieved in that HST-1 can regulate megakaryocyte development as a megakaryocyte potentiator (Meg-POT) synergistically with IL-3 and TPO as well as potentiates megakaryocyte maturation¹⁶. Therefore, it may be worthwhile to test its synergistic action with HST-1 protein on thrombocytopenia.

One advantage of the recombinant adenovirus vector is its continuous protein expression lasting at least 30 days with a single injection, which worked effectively to prevent thrombocytopenia induced by chemotherapy or irradiation in cancer treatment. Our results also have made adenovirus a general model of gene delivery for short-term therapy of

acute disorders.

HST-1 is a potent inducer of limb development

The initiation of limb development depends on the site specific proliferation of the mesenchyme by signals from the apical ectodermal ridge (AER) in embryonic mouse. We have previously reported that the local expression of *HST-1/FGF-4* transcripts in AER of the mouse limb bud is developmentally regulated, expressed at 11 and 12 days post coitus (p.c.) embryo¹⁷. In an effort to further understand the role of HST-1/FGF-4 in mouse limb development, an antisense oligodeoxynucleotides (ODNs) study was performed.

We first established a novel organ culture system to study mouse limb development *in vitro*. This system allows mouse

Fig. 2. HST-1 knockout mice are embryonic lethal : Left panel shows schematic representation of targeting *HST-1* gene in ES cells. Right panel, upper, shows phenotype of heterozygote of targeted mice at day 14.5 embryos. Right panel, lower, shows HST-1 genotypes of embryos derived from heterozygote intercrosses.

Table 1. Effects of HST-1 Antisense ODNs on P-D Length of Cultured Limbs

ODNs	Length of Limb in mm (mean±S.D.)	
	Forelimbs	Hindlimbs
None	1.312±0.04	1.642±0.14
Antisense	0.522±0.30	0.688±0.10
Sense	1.300±0.05	1.598±0.04
Scrambled	1.295±0.20	1.615±0.10

Trunc fragments with limbs (9.5–10 days p.c. embryo) were treated with ODNs as described and cultured on matrix gel for 4.5 days in limb differentiation medium. Measurements of the Proximal-Distal (P-D) length were made for each pair of limbs. Number of forelimb or hindlimb pairs analyzed=25.

limb bud at 9.5–10 days p.c. embryo—when placed on a sheet of extracellular matrix in a defined medium—to differentiate into a limb at 12.5 days p.c. embryo within 4.5 days. This culturing system not only allows limb outgrowth but also provides us with a suitable model system to explore the key factors for limb development by controlling a variety of culture conditions with or without additives such as several growth factors, inhibitors, synthetic peptides, and ODNs. Using this organ culture system, we have shown that exposure of 9.5–10 days p.c. embryonic limb bud explants to antisense ODNs of HST-1/FGF-4 blocks limb development (Table 1)¹⁸. In contrast, sense and scrambled ODNs have no inhibitory effect on limb outgrowth. These results suggest that HST-1/FGF-4 may work as a potent inducing factor for mouse limb development. Similar antisense strategies can possibly be used to clarify the functions of many other growth factors/cytokines and their receptors on embryonic limb development by using our novel mouse limb culture system.

Mice with disrupted *HST-1* gene are embryonic lethal

Fibroblast growth factors are believed to play many distinct roles in vertebrate development, due to their ability to stimulate cell growth, determine cell fate and inhibit terminal differentiation in a variety of *in vitro* cell culture systems. Among them, HST-1/FGF-4 gene expression *in vivo* is spatially restricted within many tissues including morula (E3.3–4), primitive streak (E7.5–8.5), paraxial presomitic mesoderm in the trunk (E7.5–11.5), primitive neuro ectoderm (E8.0–8.5), pharyngeal pouch endoderm (E8.5–9.5), branchial arch ectoderm (E8.5–9.5), limb apical ectoderm (E11–12), and skeletal myoblast groups (E9.5–13.5)¹⁹. However, the *HST-1/FGF-4* gene is normally silent in adult animals, and little is known about the biological significance of the HST-1/FGF-4 in embryogenesis. In an effort to better understand the role of HST-1/FGF-4 gene in murine development, we embarked on a gene-targeting experiment aimed at generating mice lacking a functional HST-1/FGF-4 gene.

Although heterozygous males and females showed significant developmental delay during embryonic days 10.5 to 18.5, they were phenotypically normal and fertile after birth. Homozygous HST-1 mutants showed severe developmental delay and died before 9 days of gestation (Fig. 2), suggesting that HST-1 plays essential roles in early phases of mouse development, in addition to its crucial role in limb development. Our results are in line with the knockout mice of FGF-4 generated by Goldfarb *et al*²⁰.

Summary

Gene knock-in (gain of function) and knock-out (loss of function) were performed to explore a novel function of the *HST-1* gene *in vivo*. Adenovirus vectors which were used for systemic expression of the target gene (gene knock-in) allowed identification of the target cells and tissues which are susceptible to the biological actions of HST-1 protein *in vivo*. Antisense technology and the gene targeting method facilitated the elucidation of the significance of the *HST-1/FGF-4* gene in early and late embryogenesis.

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