Novel immunologic tolerance of human cancer cell xenotransplants in zebrafish

BEIBEI ZHANG, YASUHITO SHIMADA, TOMOKAZU HIROTA, MICHIKO ARIYOSHI, JUNYA KUROYANAGI, YUHEI NISHIMURA, and TOSHIO TANAKA

MIE, JAPAN

Immune deficiency or suppression in host animals is an essential precondition for the success of cancer cell xenotransplantation because the host immune system has a tendency to reject implanted cells. However, in such animals, the typical tumor microenvironment seen in cancer subjects does not form because of the lack of normal immunity. Here, we developed a novel zebrafish (*Danio rerio*) model based on 2 rounds of cancer cell xenotransplantation that achieved cancer-specific immunologic tolerance without immunosuppression. We irradiated human cancer cells (PC-3, K562 and HepG2) to abolish their proliferative abilities and implanted them into zebrafish larvae. These cells survived for 2 weeks in the developing host. Three months after the first implantation, the zebrafish were implanted with the same, but nonirradiated, cell lines. These cancer cells proliferated and exhibited metastasis without immune suppression. To reveal the transcriptional mechanism of this immune tolerance, we conducted dual RNA-seq of the tumor with its surrounding tissues and identified several regulatory zebrafish genes that are involved in immunity; the expression of *plasminogen activator, urokinase*, and *forkhead box P3* was altered in response to immunologic tolerance. In conclusion, this xenograft method has potential as a platform for zebrafish-based anticancer drug discovery because it can closely mimic human clinical cancers without inducing immune suppression. (Translational Research 2016;170:89–98)

Abbreviations: cd3 = cd3ζ; cDNA = complementary DNA; CML = chronic myelogenous leukemia; dpf = day post fertilization; e2f1/3 = E2F family of transcription factor 1 and 3; foxp3 = forkhead box P3; hpf = hour post fertilization; il-17 = interleukin 17; ifn-γ = interferon gamma; IFNG = interferon gamma; klf1 = Kruppel-like factor 1 (erythroid); KOr = Kusabira-Orange; LPS = lipopolysaccharide; mRNA = messenger RNA; mpf = month post fertilization; myod1 = myogenic differentiation 1; MEK1/2 = mitogen-activated protein kinase kinase 1/2; NIH = National Institutes of Health; RAS/RAF/MEK = Rat sarcoma/Rapidly Accelerated Fibrosarcoma/Mitogen-activated protein kinase; SEM = standard error of the mean; SNEA = subnetwork enrichment analysis; STAT5A = signal transducer and activator of transcription 5A; tbx20 = T-box 20

From the Department of Molecular and Cellular Pharmacology, Mie University Graduate School of Medicine, Mie, Japan; Department of Pharmacogenomics and Pharmacoinformatics, Mie University Graduate School of Medicine, Mie, Japan; Department of Systems Pharmacology, Mie University Graduate School of Medicine, Mie, Japan; Mie University Medical Zebrafish Research Center, Mie, Japan; Department of Bioinformatics, Mie University Life Science Research Center, Mie, Japan; Department of Omics Medicine, Mie University Industrial Technology Innovation Institute, Mie, Japan.

B. Zhang and Y. Shimada contributed equally to this work.

Submitted for publication July 27, 2015; revision submitted November 22, 2015; accepted for publication December 14, 2015.

Reprint requests: Toshio Tanaka, Department of Molecular and Cellular Pharmacology, Pharmacogenomics and Pharmacoinformatics, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan; e-mail: tanaka@doc.medic.mie-u.ac.jp.

1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2015.12.007

AT A GLANCE COMMENTARY

Zhang B, et al.

Background

Xenotransplantation of human cancer cells provides a valuable model for cancer research. Because the host immune system has a tendency to reject implanted cells, immune deficiency or suppression is an essential precondition. However, in such animals, the typical tumor microenvironment does not form because of the lack of normal immunity.

Translational Significance

We developed a novel zebrafish xenograft model based on a 2-round cancer cell implantation strategy that achieved cancer-specific immunologic tolerance without immunosuppression. Dual RNA-seq analysis revealed that this xenograft protocol can closely mimic human clinical cancers. This model offers a useful platform for the investigation of cancer progression.

INTRODUCTION

Xenotransplantation of human cancer cells provides a valuable model for pathophysiological and therapeutic studies in cancer research. One challenge is the avoidance of immune rejection by the host animal. Induction of immune deficiency, for example using the NOD/SCID mouse,[1] γ-irradiation, or immune-suppressing agents,[2] has been used to facilitate xenotransplantation. Although these models have contributed greatly to the understanding of human cancers, the lack of systemic immunity, mainly T- and B-cell function, is a problem for further understanding of cancer mechanisms.[3,4] Correspondingly, the drug response in cancer xenografted "nude" animals does not always correlate to the clinical situation owing to the changes in the immune system.[5] In addition, the immune suppression treatments can induce spontaneous tumors in the host, thus confounding xenotransplantation cancer studies.[6]

Zebrafish (*Danio rerio*) has been successfully used for decades as a model organism for preclinical studies and drug discovery. In cancer research, cancer xenograft zebrafish represents a promising alternative model to study tumor proliferation,[7,8] angiogenesis,[9,10] metastasis,[11,12] and cancer stemness.[13,14] To avoid immune rejection of the xenograft, cancer cell implantation has been performed at the embryonic stage (24–72 hours postfertilization, hpf) when the adaptive immune response is not established. Although the sites of hematopoiesis are different between zebrafish and mammals, the cellular and regulatory processes of hematopoiesis, including for immune cells, are highly conserved.[15,16] In fact, most of the immune cells found in mammals have also been identified in zebrafish.[17,18] Because of this similarity, the number of studies examining adult zebrafish xenografts is small. Gamma irradiation[19] and immune suppressants[20] have been used to ablate immune cells of zebrafish to establish adult zebrafish xenografts. Recently, Tang et al. developed an immunocompromised zebrafish model using *rag2* mutants, which allows long-term engraftment of human cancer cells.[21] In spite of these successful studies, such methodologies are limited owing to the lack of a normal immune system in the host.

In this study, we established a novel cancer xenograft zebrafish with immunologic tolerance specific to the implanted cancer cells. The implanted cancer cells proliferated and exhibited metastasis without host immune suppression.

MATERIALS AND METHODS

Ethical approval. The animal experiments in this article conformed to the ethical guidelines established by the Institutional Animal Care and Use Committee of Mie University, which follow the NIH guidelines (Guide for the Care and Use of Laboratory Animals).

Cell culture and fluorescent labeling. PC-3 cells (human prostate cancer), K562 cells (human chronic myelogenous leukemia), and HepG2 cells (human hepatocarcinoma) were obtained from the RIKEN Cell Bank (Tokyo, Japan). The methods for cell culture and fluorescent protein labeling were performed as in a previous study.[22] We denoted *Kusabira-Orange* (KOr) protein-expressing cells as PC3-KOr, K562-KOr, and HepG2-KOr, respectively.

γ-Irradiation of cancer cells. PC3-KOr, K562-KOr, and HepG2-KOr cells were cultured in 25-cm^2 flasks containing 10-mL medium at a density of 4×10^5 cells/mL. Irradiation was delivered from a cobolt-60 gamma irradiation using a CAX-150-20 (Chubu Medical, Aichi, Japan). We prepared 10 Gy of irradiated PC3-KOr, 6 Gy of irradiated K562-KOr, and 10 Gy of irradiated HepG2-KOr cells; all of which lacked proliferative capacity but did not die. We denoted the above irradiated cells as Ir-PC3-KOr, Ir-K562-KOr, and Ir-HepG2-KOr, respectively.

Zebrafish. Care and breeding of zebrafish were performed following protocols as described by Westerfield.[23] *nacre/rose/fli1:egfp* zebrafish was used because