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14. ABSTRACT

We plan to study the consequences of BCR-ABL expression in myeloid cells, using the zebrafish model system. The fundamental genetic mechanisms that control hematopoiesis are well conserved, and many of the genes known to regulate these processes in mammals have been recently identified in zebrafish. Advantages of the zebrafish provide an unparalleled opportunity to identify specific genes whose mutational inactivation blocks BCR-ABL activity, and whose products therefore serve as potential targets for the development of small molecule inhibitors that could be used in treatment of CML. Determination of the patho-physiologic significance of BCR-ABL in transgenic zebrafish models and stable transgenic zebrafish models for BCR-ABL induced leukemia will establish the necessary groundwork that will be valuable in conducting future modifier screens.

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Introduction

The goal of our proposal (CM030067; Zebrafish models of BCR-ABL induced leukaemogenesis) is to determine the pathophysiologic significance of BCR-ABL in transgenic zebrafish models, establishing the necessary groundwork that will be valuable in conducting future modifier screens.

It is generally believed that Chronic Myelogenous Leukemia (CML) develops when a single, pluripotential, hematopoietic stem cell acquires a Ph chromosome carrying the BCR-ABL fusion gene, conferring a proliferative advantage over normal hematopoietic elements and allows the Ph-positive clone gradually to displace residual normal hematopoiesis (Eaves et al. 1997, 2001). CML generally requires allogeneic bone marrow transplantation for cure, but efforts to extend this form of treatment to all patients with CML have been thwarted by the lack of suitable donors and risk of potentially lethal graft-versus-host disease (GVHD). The recent introduction into clinical practice of a tyrosine kinase inhibitor (imatinib) that specifically blocks the enzymatic action of the BCR-ABL fusion protein promises to be a major contribution to the management of CML. However, no information on long-term survival with imatinib is available yet. Futhermore, observation of imatinib resistance even in chronic phase (Hochhaus et al. 2002) and of residual disease in complete cytogenetic responders, casts doubt on the life prolonging potential of imatinib monotherapy.

Studies in zebrafish models complement research in human cell lines and mouse models because of the inherent capacity of zebrafish to accommodate genome-wide enhancer-suppressor screens for modifier genes in critical pathways that exacerbate abnormal myelopoietic phenotypes or restore normal hematopoiesis. This advantage provides an unparalleled opportunity to identify specific genes whose mutational inactivation blocks BCR-ABL activity, and whose products therefore serve as potential targets for the development of small molecule inhibitors that could be used in treatment of CML.

Body

1. We hypothesized that the expression of BCR-ABL in zebrafish embryos under the control of a myeloid cell specific promoter will cause abnormal proliferation and expansion of the number of granulocytes and their progenitors through changes involving cell growth, viability and differentiation. We will take advantage of the zebrafish model system to directly visualize myeloid progenitors in the developing embryo in vivo, through the analysis of transgenic zebrafish microinjected (as fertilized eggs) with a BCR-ABL protein.

2. We hypothesized that stable lines of transgenic zebrafish expressing BCR-ABL will develop hypercellular kidney marrow, splenomegaly, and neutrophilia resembling a myeloproliferative disorder. The development of stable transgenic lines is a critical step in the future goal of conducting modifier screens to discover additional important genes in the oncogenic pathway used by BCR-ABL. Insight into this pathway may lead to the identification of modifier genes that can serve as potential drug targets.

Objectives:
Aim 1. To define the spectrum of phenotypes, including a potential proliferative and survival advantage, induced by constitutive expression of BCR-ABL in developing myeloid cells in vivo.

Aim 2. Establish stable transgenic lines of zebrafish overexpressing BCR-ABL in myeloid stem and progenitor cells and monitor these fish for the development of myeloproliferative syndrome and AML.

Key Research Accomplishments

The zebrafish *pu.1* promoter fragment we have isolated and used to generate a *zpu.1-GFP* transgenic line is active not only in myeloid progenitor cells but also in the trunk musculature. This ectopic expression will not pose a problem for our investigations. However, in the *zpu.1-GFP* transgenics, GFP is also detectable at the one cell stage of fertilized embryos indicating maternal contribution (Hsu et al. 2004). A similar maternal expression of GFP has been detected using an *mpo* promoter (*FmpoP*) fragment isolated from the pufferfish *takifugu rubripes* (Grabher, *unpublished*). Previous experience from our lab and others has demonstrated that such an early expression of a highly potent oncogene such as BCR-ABL is lethal to the animals before or during gastrulation.

For optimal time management we have thus decided to start preparations for Aim 2 before engaging Aim 1. In order to avoid an early lethal BCR-ABL phenotype, we designed a conditional vector applying the *cre-lox* system that has been established for zebrafish in our laboratory (Langenau et al. 2005). In order to be able to exchange different promoter elements easily, we have adapted the GATEWAY cloning system (Invitrogen). Adaptation of this highly efficient cloning system was tedious but was successful. So far we succeeded in designing a conditional vector containing a *FmpoP-lox-GFPpA-lox-BCR-ABLpA* expression cassette. This entire expression cassette is flanked by two I-SceI meganuclease restriction sites, which will increase transgenesis frequencies when co-injected with the I-SceI enzyme (Grabher et al. 2004). We successfully injected several hundred embryos with this vector and have pre-screened them for promising putative founder fish based on GFP expression. Approximately, one hundred putative founder fish are currently growing and will reach sexual maturity within the next month when we will start screening for transgene transmission to the offspring to identify germline founders. We are currently generating the same vector containing the *zpu.1* promoter fragment in place of the *FmpoP* to establish additional transgenics driving BCR-ABL under the control of this promoter upon excision of the lox-GFPpA-lox cassette by injection of *cre* mRNA into one-cell stage transgenic embryos or mating of BCR-ABL transgenics to a heat-shock inducible *cre* transgenic line.
Following injections of several hundred embryos with *zpu.1-lox-GFPpA-lox-BCR-ABLpA* vector and prescreening for promising putative founders, we will engage in the transient experiments described as Aim 1 in the original proposal.

Reportable Outcomes

We have successfully designed conditional expression vectors using two different promoter fragments (FmpoP and zpu.1) to drive BCR-ABL in a conditional manner using the *cre-lox* system. Furthermore we are in the process of generating stable transgenic lines with both of these transgenic vectors.

Conclusions

We are on track for generating stable transgenic animals expressing BCR-ABL in a conditional manner. We hypothesize that stable lines of transgenic zebrafish expressing BCR-ABL will develop hypercellular kidney marrow, splenomegaly, and neutrophilia resembling a myeloproliferative disorder. Lines expressing BCR-ABL in myeloid cells are necessary for the long-term goal of conducting modifier screens to discover novel important genes that are essential to the oncogenic pathways used by BCR-ABL.
While putative founder fish are growing we will initiate the transient studies of Aim 1.

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