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Leukemia Research 29 (2005) 803–812
Leukemia Research
www.elsevier.com/locate/leukres

Wt1 is not essential for hematopoiesis in the mouse

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Received 3 June 2004; accepted 26 November 2004
Available online 17 February 2005

Abstract

WT1 has been implicated in human leukemia and hematopoiesis, but its role in stem cell differentiation is not yet fully defined. We show that Wt1-null murine fetal liver cells are capable of reconstituting functional hematopoiesis following transplantation into irradiated recipients. There was also no significant difference between the in vitro colony-forming ability of wild-type and Wt1-null cells. Using a reporter gene assay in a transgenic mouse system, expression from the *WT1* promoter was detectable in adult bone marrow, but undetectable in subsets of different hematopoietic cells. We conclude that Wt1 is not essential for murine hematopoiesis and that there may be significant differences in its role between mouse and man.

Keywords: Wt1; Hematopoiesis; Mouse; Leukemia; Transplantation; Stem cells

1. Introduction

WT1 was originally identified as a gene involved in predisposition to the childhood kidney cancer Wilms tumor and is a paradigm for the relation of normal developmental processes to tumorigenesis [1]. The gene encodes a zinc finger protein that can function as a transcription factor and may also be involved in RNA processing [2]. Expression of WT1 is highest during embryogenesis, where it is found in multipotent progenitor cells of a restricted range of tissues, mainly in the genitourinary system [3]. In adults, expression continues in specific cell types of the kidney and gonad and, at much lower levels, in the bone marrow, where it is confined to CD34+ progenitor cells [4–7]. In murine hematopoiesis, expression of Wt1 has been reported at a low level in the fetal liver and yolk sac, and in adult bone marrow and blood by reverse transcriptase-polymerase chain reaction (RT-PCR) [8].

As in Wilms tumors, the majority of acute leukemias express a high level of WT1 [4,9], and *WT1* mutations can be found in 10–15% of cases [5,10,11]. In both Wilms tumor and leukemia, the high level of expression may simply reflect the primitive cell of origin of the tumor, rather than being a contributing factor towards the progression of tumorigenesis. Alternatively, a high level of WT1 expression may confer a growth or survival advantage to tumor cells by preventing or delaying the response to differentiation signals. This effect has been demonstrated in a number of WT1-expressing leukemic cell lines [12–14] and in hematopoietic progenitor cells [15,16].

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doi:10.1016/j.leukres.2004.11.020

Although *WT1* mutations occur mainly in acute myeloid leukemias, they are also found in undifferentiated, biphenotypic and lymphoblastic leukemias, suggesting a role for WT1 in very early hematopoiesis, prior to determination of the lymphoid/myeloid split. This is supported by data showing that the highest level of WT1 expression is seen in leukemias with immature phenotypes [17], and that WT1 is expressed at sites where hematopoietic stem cells are known to reside during embryogenesis. Expression of WT1 is downregulated during differentiation of leukemic cell lines and high levels of WT1 expression can cause cell cycle arrest and/or apoptosis [9,18]. In normal hematopoiesis, WT1 is expressed in quiescent primitive progenitors and in some differentiated cells but not in the intermediate lineage-committed precursors [19]. This is similar to the situation in kidney development where WT1 expression rises to a peak during glomerular differentiation, declines and then persists only in podocyte cells. However, the function(s) of WT1 in hematopoietic differentiation is not yet understood, although possible target genes for transcriptional regulation such as colony-stimulating factor have been identified in vitro [20]. There are hematopoietic-specific mechanisms of controlling WT1 function, such as tissue-specific enhancers within the *WT1* gene [21,22] and variations in exon 5 splicing [23], which suggest that WT1 is important in hematopoietic cells.

The Wt1 knockout mouse demonstrates that the gene is essential for the formation of kidneys, gonads and spleen [24,25]. The absence of renal development is attributable to the loss by apoptosis of metanephric blastemal cells that normally express a low level of Wt1 [24]. Wt1 is also expressed in the aorta-gonad-mesonephros (AGM) region [26], the site where hematopoietic stem cells first arise during embryogenesis, and in the fetal liver [8], but there is no obvious hematopoietic defect in heterozygous null mice, and homozygous Wt1-null mice die in utero [24] or at birth [25].

Given that WT1 clearly has a role in leukemia, but that its importance in hematopoiesis is still rather obscure, we sought to analyse its expression and function with the use of two transgenic mouse models. To study the expression of Wt1 in fetal and adult hematopoiesis we used a WT1 *lacZ* fusion transgenic line, which contains the *E. coli lacZ* gene under the control of the human *WT1* promoter. This *YAC* transgene mimics the endogenous expression of the *Wt1* gene during embryogenesis [26]. Furthermore, the same YAC containing the intact *WT1* gene can be used to complement the Wt1-null phenotype [27]. Since fetal liver is a rich source of hematopoietic stem cells (HSCs) [28,29], we undertook transplantation of liver cells from donor Wt1-null embryos into lethally irradiated recipient animals in order to assess whether Wt1 is required for reconstitution of hematopoiesis. In addition, hematopoietic progenitor cells derived from the AGM region of Wt1-null embryos were placed in culture to determine their ability to differentiate into the full range of hematopoietic lineages.

2. Materials and methods

2.1. WT1-β-galactosidase transgenic mouse lines

Line H (WT470LZ^{h}) mice were generated as described [26]. The transgene was derived from WT470, a 470 kb YAC spanning the human *WT1* locus. A *lacZ* reporter gene was introduced into WT470 by homologous recombination, resulting in an in-frame fusion of *WT1* exon 1 and the *lacZ* gene (WT470LZ), and placing expression of *lacZ* under the control of the human *WT1* promoter. Line H contains nine copies of WT470LZ, all of which are integrated near the telomere of chromosome 4. The presence of the transgene in mice was determined by PCR on DNA obtained from ear cuffs using primers specific for bacterial *lacZ* as previously described [26].

2.2. Determination of β-galactosidase activity by staining with X-gal

The number of β-galactosidase positive cells in total adult bone marrow (BM) was determined by staining with X-gal (5-bromo-4-chloro-3-indolyl β-D-galactosidase, Sigma). Staining was carried out in 24-well culture dishes as described [30], with an additional fixation in 4% paraformaldehyde for 15 min after X-gal staining. The genotype of each animal was reconfirmed at the time of BM harvest by staining frozen kidney sections with X-gal. In order to assess β-galactosidase activity during early stages of myeloid or macrophage differentiation, transgenic BM cells were plated onto soft agar in α-MEM containing either 5% spleen-conditioned medium (containing GM-CSF and IL-3), or 100 ng/ml M-CSF. Colonies were stained as above over 14 days.

2.3. Determination of β-galactosidase activity by flow cytometry

The β-galactosidase activity in viable transgenic BM mononuclear cells was analyzed using a Fluoreporter® lacZ flow cytometry kit (Cambridge Bioscience, Cambridge, UK) and an antibody to β-galactosidase. The BM cells were fixed and permeabilized at room temperature using a cell permeabilization kit (Harlan SERA-LAB), and then washed in PBS prior to incubation with a monoclonal anti-β-galactosidase-biotin conjugate (Sigma) diluted to 1:100 in PBS/2.5% FCS, or with a biotinylated IgG1 isotype control (DAKO Ltd.) for 30 min at 4 °C. The cells were washed in PBS/2.5% FCS prior to incubation with fluorescein streptavidin (Vector Laboratories) diluted to 1:100 for 30 min at 4 °C. Samples were analyzed using an EPICS Elite ESP cell sorter (Beckman Coulter, High Wycombe, UK) and Coulter's operating system software. The positive control cell lines used were a polyPOZ virus producer line [31] and Flp-In™-293 (Invitrogen).