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DNA-binding dependent and independent functions of WT1 protein during human hematopoiesis

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Abstract

The Wilms tumor gene 1 (WT1) encodes a zinc-finger-containing transcription factor highly expressed in immature hematopoietic progenitor cells. Overexpression and presence of somatic mutations in acute leukemia indicate a role for WT1 in the pathogenesis of leukemia. $CD34^+$ progenitor cells were transduced with one splice variant of human WT1 without the KTS insert in the zinc-finger domain, WT1(+/−), and with a deleted mutant of WT1 lacking the entire zinc-finger region, WT1(delZ), thus incapable of binding DNA. We show that inhibition of erythroid colony formation and differentiation is absolutely dependent on the DNA-binding zinc-finger domain of WT1. Unexpectedly, however, WT1(delZ) was equally effective as wild type protein in the reduction of myeloid clonogenic growth as well as in stimulation of myeloid differentiation, as judged by the expression of cell surface CD11b. Expression of neither WT1(+/−) nor WT1(delZ) upregulated mRNA for the cdk inhibitor $p21^{Waf1/Cip1}$ or $p27^{Kip1}$. Our results demonstrate that WT1 affects proliferation and differentiation in erythroid and myeloid cells by different molecular mechanisms, and suggest that mutations affecting the zinc-finger domain of WT1 could interfere with normal differentiation in the pathogenesis of leukemia.

Keywords: Wilms tumor gene 1; Leukemia; Hematopoiesis; Zinc-finger; Differentiation

Introduction

The Wilms tumor 1 (WT1) protein is a zinc-finger transcription factor, the somatic loss of which confers increased risk for development of Wilms' tumor [1,2]. The dependence on WT1 for the development of certain organs is evident in mice with homozygous inactivation of WT1, leading to embryonic lethality at days 13–14 as a result of severe malformations of gonads, kidneys, liver, spleen, heart, and mesenchymal structures [3]. Given that homozygous inactivation of WT1 leads to early embryonic lethality, that model does not provide much information regarding the consequences of loss of WT1 for adult hematopoiesis or development of hematopoietic malignancy. Using chimeric mice and in vivo repopulating assays, however, it was recently shown that hematopoietic stem cells and progenitors lacking WT1 compete poorly with their normal counterparts [4]. Moreover, heterozygosity for WT1 in mice predisposes to spontaneous development of lymphoma [5]. Thus, the present data support a role for WT1 in hematopoiesis in vivo.

A developmental role for WT1 in the human hematopoietic system is indicated by a number of observations. WT1 is expressed in a small subset of dormant as well as lineage-committed progenitor cells, but is not detectable in peripheral mature blood cells [6–9]. Most acute leukemias show high expression of WT1, the level of which correlates to poor outcome [10–15], and although the number of analyzed cases in the literature is low, AML with heterozygous mutations of WT1 might be associated with a poor response to chemotherapy [16–19], arguing for effects of WT1 on viability, proliferation, and differentiation. Results from forced expression of WT1 in leukemic cell lines,

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showing interference with the differentiation response [20–25] and the observation that murine bone marrow cells transduced with human WT1 showed decreased differentiation under certain culture conditions [26], lend further support for the notion of WT1 having a functional role in the differentiation of hematopoietic tissues.

Recently, forced expression of WT1 by retroviral infection of human hematopoietic progenitor cells was reported, demonstrating anti-proliferative effects on myeloid and in particular on erythroid clonogenic cells [27,28]. No impairment of the differentiation into mature cells was observed, but rather an enhanced differentiation of committed myelomonocytic progenitors was indicated [27,28]. The mechanisms for the observed effects are not explained, but expression of the cdk inhibitor $p21^{Waf1/Cip1}$ (p21), a defined target gene for WT1, was not alone sufficient for the effects on proliferation and differentiation exerted by WT1 [28], indicating involvement of other transcriptional targets or direct interactions of WT1 with other proteins. The molecular mechanisms by which WT1 affects hematopoietic proliferation and differentiation are therefore presently unclear.

The WT1 protein is a transcription factor; its DNA-binding domain consists of four carboxyl terminal zinc-fingers while an aminoterminal proline–glutamine-rich region has transcriptional regulatory activity [29,30]. Alternative splice variants include or exclude 17 amino acids close to the zinc-fingers (±17AA) and a 3-amino acid (lysine–threonine–serine) insert between zinc-fingers 3 and 4 (±KTS) [31]. The 17AA sequence, encoded by exon 5, can mediate binding to other transcriptional co-factors such as Par4 [32], but transgenic mice lacking exon 5 show no obvious developmental defects [33]. Similarly, mice homozygous for WT1 lacking the initial 68 aminoterminal amino acids develop normally into adulthood [34]. The zinc-finger domain of WT1 mediates binding to several different DNA motifs in promoters: a TCC-repeat, the DNA-binding site for early growth response 1 protein (EGR1), and the WTE-site, which is similar to the EGR1-site [35,36]. It is generally believed that WT1(-KTS) has a broader target site specificity than WT1(+KTS) [37–40], and different nuclear localizations of the two WT1 isoforms support the notion of at least partly different functional roles [41,42]. The zinc-fingers of WT1 also mediate binding to certain other proteins such as p53 and p73, potentially altering the transactivating properties of WT1 [43,44].

Mutations of WT1 are observed in adult as well as childhood leukemia, most common in AML. In four studies, in total investigating more than 100 leukemia samples, mutations in WT1 were found in 10–12% of the cases [16–19]. AML-associated mutations of WT1 are most often small insertions causing frameshifts or missense mutations, encoding WT1 proteins with deletions of most of the Zn-finger domain or with amino acid substitutions in the zinc-fingers, respectively. Heterozygous point mutations in the zinc-finger region, potentially affecting binding to DNA, underlie the congenital malformation syndromes WAGR and DDS [45]. Both WAGR and DDS predispose for the development of Wilms' tumor, but while the mutations are dominant for developmental disturbances, they are most often recessive for tumor development, arguing for dominant functions in the first case and loss of function in the latter [45]. While some cases of AML show homozygous or compound mutations of WT1, the majority of WT1 mutations in leukemia are heterozygous, suggesting a dominant-negative effect or acquisition of novel gain-of-function of mutant WT1 in leukemogenesis.

Some observations indicate that WT1 may exert transcriptional regulation independently of DNA-binding; like full-length WT1, the aminoterminal half of WT1 did partially repress the promoter for insulin-like growth factor I [46], and overexpression of WT1 with a missense mutation in the zinc-fingers inhibits growth of tumor cells in vivo equally well as full-length WT1 protein [47]. Since WT1 with impaired DNA-binding in these cases has similar effect as full-length WT1, it cannot act by a dominant-negative effect on full-length WT1. The mechanism by which the aminoterminus exerts its effects is therefore unknown, but could involve RNA–protein interactions; the aminoterminal WT1 contains a postulated RNA-binding motif [48]. In other reports, however, the zinc-finger domain of WT1 mediates RNA-binding [49]. Alternatively, aminoterminal WT1 might be interacting with other proteins, affecting transcription without direct DNA-binding. For example, CAAT-boxes may respond to WT1 even though WT1 does not bind directly to that element [50,51] and WT1 can bind to p53 thereby affecting transcription from promoters containing p53 response elements [43]. Most protein interactions of WT1 with other proteins, e.g., p53, p73, and CBP, are mediated by the zinc-fingers [43,44,52], but binding of certain proteins, including WT1 itself, is mediated by the aminoterminus [30,53]. Interestingly, the subnuclear localization of aminoterminal WT1 is distinct to that of full-length WT1 [42]. Therefore, aminoterminal WT1 may interact with proteins distinct from those normally exposed to full-length WT1.

Given that most AML-associated mutations of WT1 affect the zinc-finger domain, the aim of the present work was to investigate potential effects of WT1 lacking zinc-fingers on hematopoietic differentiation and proliferation. To this end we have overexpressed human WT1 and a mutant form of WT1, lacking the entire zinc-finger region, in human hematopoietic progenitor cells. We show that negative effects on erythroid differentiation and clonogenic capacity are entirely dependent on the zinc-finger domain, but that anti-clonogenic effects and pro-differentiation effects on myeloid progenitors are efficiently exerted by mutant WT1. We conclude that WT1 can affect myeloid and erythroid hematopoiesis by distinct mechanisms and that mutated WT1 may affect hematopoiesis not only by dominant-negative effects.