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The Role of *dead-end* in Germ-cell Tumor Development

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Abstract

Testicular germ-cell tumors occur in human males of all age groups, from infants to men over 50 years old. Most commonly, germ-cell tumors (generally known as testicular cancer) occur in young males between the ages of 15 to 35 years. The tumor tissues are usually histologically diverse, and testicular tumors that occur in the different age groups tend to be of specific histological subtypes. Most germ-cell tumors originate from primordial germ cells during embryonic development, although the progression and eventual detection of the disease occurs decades later in humans. Mouse strains spontaneously develop a specific subtype of testicular germ-cell tumors, the type I germ-cell tumors, and these tumors are similar to the germ-cell tumors (or teratomas) that occur in human infants. Some mouse strains, such as the 129-*Ter* strain, have extremely high germ-cell tumor incidences, making such strains ideal for genetic and biological studies of germ cell-tumor development. Here a brief overview of the recently identified genetic defect in the *Ter* strain, inactivation of the *dead-end* (*Dnd1*) gene, and the ongoing studies on *Dnd1* to understand its role in germ-cell and germ cell-tumor development, are provided.

Keywords

dead end; Dnd1; Ter; testicular germ-cell tumors; 129 inbred strain

Teratomas are tumors that histologically appear as a random assortment of embryonic and differentiated adult tissues. Teratomas in mice can be experimentally generated to appear at various sites in the animal,^{1,2} or they may develop spontaneously in the gonads of specific mouse strains.³ When embryonic stem cells are transplanted to ectopic sites in adults, they will develop into teratomas. Experiments carried out by Leroy Stevens showed that when he transplanted genital ridges (or embryonic gonads dissected out from mouse embryos) under the testis capsule, these transplantations developed into teratomas.^{1,2} He then showed that the teratomas develop only when the genital ridges have normal primordial germ cells (PGCs), and that teratomas develop only when the genital ridges are derived from mouse embryos no later than embryonic day (E) 13.5.⁴ This indicated that mitotically proliferating primordial germ cells present in the genital ridges originated the teratomas.

Stevens also found that the 129 inbred mouse strain spontaneously develops teratomas in the testes.³ Spontaneous tumor development is restricted to a few inbred mouse strains. About 10% of the males of the 129 strain develop spontaneous testicular tumors (or teratomas). The rest of the males appear normal and are fertile. Also, the tumors in 129 are generally unilateral, that is, they only develop in one testis of the male, whereas the other testis is normal. In the 1970s, a fortuitous mutation arose in Stevens' 129 mouse colony. This mutation caused an increased incidence of testicular teratomas in mice such that 94% of the males developed

testicular tumors.^{5,6} Also, 75% of the tumors were bilateral, that is, affecting both testes. The isolation of this mouse strain, named *Ter* (for teratomas), with its very high testicular teratoma incidence, has allowed for more extensive studies to be carried out regarding the etiology and genetics of spontaneous testicular-tumor development.

Examination of the 129-*Ter* mice revealed that tumor development in the 129-*Ter* strain starts around E12.5.^{7,8} For reasons not understood, PGCs are first transformed into embryonal carcinoma (EC) cells. These EC cells proliferate rapidly. EC cells are readily observed in the gonads after E16.5.^{7,8} The EC cells proliferate within the developing seminiferous cords and eventually rupture the basement membrane of the testis cords to invade the interstitial spaces. The EC cells continue to proliferate within the gonads until after birth. However, approximately 5 days after birth, most EC cells differentiate randomly into adult and embryonic tissues that constitute the teratomas (Fig. 1D). Thus, spontaneous teratomas in the testis of mice are visible by eye soon after birth. These tumors are similar to testicular type 1 germ-cell tumors that occur in human infants.⁹

Further studies showed that the *Ter* defect causes tumors only on the 129 background. When *Ter* is transferred onto other mouse-strain backgrounds, few tumors were observed. However, the mice were sterile at birth and had small gonads.⁶ Examination of primordial germ-cell development revealed that the primary defect in the *Ter* mouse strain is progressive loss of PGCs after E8.5¹⁰ on all strain backgrounds. Thus, all mice homozygous for the *Ter* defect are sterile and mice with the *Ter* defect on the 129 strain background showed loss of germ cells as well as tumor development. This is illustrated in Figure 1, which shows a postnatal day 1 (PN1) testes from the 129-*Ter/Ter* strain. The transgene carrying the green fluorescent protein (GFP) driven by the germ cell-specific promoter *Oct4* (*GOF-1/ΔPE/EGFP*)^{11,12} was introduced into the 129-*Ter* strain. This strain has been described previously.¹³ In normal PN1 testes, germ cells are clearly observed because they express GFP.¹⁴ However, in PN1 testes from the 129-*Ter/Ter*; *Oct4*-GFP strain, no GFP-expressing germ cells are observed by confocal microscopy (Fig. 1B). Thus, by PN1, all germ cells are affected such that they either die or become transformed. A histological section through this testis indicates proliferative clusters of transformed EC cells (Fig. 1C). The EC cells differentiate into teratomatous tumors after birth (Fig. 1D).

Using positional cloning approaches, we determined that the defect in *Ter* mice is inactivation of the *dead-end* (*Dnd1*) gene, which is located on mouse chromosome 18.¹³ A point mutation (C to T) introduces a stop codon in the *Dnd1* gene of *Ter* mice, resulting in loss of expression of *Dnd1*.

Normally *Dnd1* transcripts are upregulated in primordial germ cells after E7.25, soon after primordial germ-cell specification, although before E7.25, *Dnd1* does not appear to be entirely specific to germ cells in the embryo.¹⁵ *Dnd1* transcripts and low levels of DND1 protein have also been detected in embryonal germ (EG) cells in culture.¹⁶ Expression of *Dnd1* transcripts is detected in the normal embryonic gonads of both sexes at E11.5. Thereafter, expression of *Dnd1* transcript increases in the male genital ridges, but decreases in the female genital ridges.¹³

Human *DND1* maps to chromosome 5q31.3. Chromosomal deletions in human 5q have been found in germ-cell tumor tissues and germ-cell tumor cell lines.^{17,18} However, to date, the status of human DND1 has not been examined extensively in type 1, human germ-cell tumors for the presence of genetic or epigenetic alterations. It will be important to establish whether defects in human DND1 also contribute to development of germ-cell tumors of infants.

In mice, there are two *Dnd1* encoded protein isoforms, DND1 α and DND1 β (Fig. 1E). DND1 α differs from DND1 β only at the first 45 and 33 amino acids of the N-terminus,

respectively.¹⁶ DND1 α is expressed in the early embryonic gonads and also in the testis after birth, continuing on in expression in adult testes, including in meiotic and postmeiotic adult germ cells. In contrast, DND1 β is expressed in the testes only after postnatal day 20. DND1 β is expressed in pachytene-stage spermatocytes and in round and elongated spermatids of the adult testis.¹⁶ These data indicate that DND1 α helps maintain PGC viability, and its loss causes transformation into EC cells. The role of DND1 α in adult germ cells is unclear, and whether it is also required to maintain viability of postnatal and adult germ cell remains to be established. The role of DND1 β in adult germ cells is at present also not known.

From what we know so far of the role of *Dnd1*, it appears to be important for maintaining viability of primordial germ cells in vertebrates.^{13,19} These observations also suggest that there are additional factors (or defects) in the 129 strain, which together with inactivation of *Dnd1*, cause primordial germ cells to transform. The nature of these factors is under investigation in different laboratories. Some clues can be obtained from studies on germ-cell tumors in humans. In humans, linkage analysis suggests that multiple loci, each with weak effects, and located on a number of different human chromosomes, including human chromosomes 2, 3, 12, 18, X, and Y, contribute to testicular germ-cell tumorigenesis.^{20–22} Thus, candidate genes located on orthologous regions in mouse chromosomes may also influence tumor development in 129 mice that have inactivated *Dnd1*.

A second approach that is underway is examination of RNA or proteins that interact with the *Dnd1*-encoded protein, DND1. Both isoforms of DND1 have identical RNA recognition motifs (RRM). Thus, efforts to identify candidate RNA that may bind to the RRM of DND1 are underway. In addition, among all the proteins that possess RRMs, DND1 shows the highest homology to ACF (APOBEC1 complementation factor). ACF is an essential RNA binding cofactor of APOBEC1.²³ APOBEC1 is an RNA-editing enzyme. The speculation is that DND1 may also be a cofactor that modulates the activity of some APOBEC family member. If this is correct, one hypothesis is that loss of DND1 may deregulate some critical nucleic acid editing in germ cells, thus leading to either germ-cell death or transformation. These ideas are currently under investigation.

One puzzle is why the 129 mouse strain only develops teratomas or testicular type 1 germ-cell tumors and does not develop germ-cell tumors in ectopic sites, as is common in humans infants,^{24,25} or develop the more common type 2 germ-cell tumors found in young human males.⁹ Although the answer is not known, the speculation is that this may be related to the time frame of germ-cell development in the mouse as opposed to in humans. PGC development in the embryo progresses rapidly in the mouse (within days) compared to that in humans (in months). Also, unlike in humans, postnatal germ cells begin mitotic proliferation approximately 5 days after birth and thus the time to puberty is extremely short in mice compared to that in humans. Thus, in humans, although germ-cell tumor cells originate during embryogenesis (known as carcinoma *in situ* cells),²⁶ the ensuing time period until puberty (about a decade) likely allows for the transformed germ cells to acquire additional genetic and epigenetic changes that manifest as the more aggressive and “dangerous” forms of germ-cell tumors. To experimentally study the biology of different types of germ-cell tumors, it will be useful to engineer multiple defects in mice so as to generate mouse models that develop the different types of testicular germ-cell tumors as are found in humans.

In summary, the identification of *Dnd1* as the cause of germ-cell loss and testicular germ cell-tumor (type 1) development in mice has opened up exciting new avenues for investigation into the mechanism of germ cell-tumor development.

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FIGURE 1.

(**A**) Testis from 129-*Ter/Ter*; *Oct4*-GFP strain postnatal day 1 (PN1) testes. PN1 testes observed using reflected light under the stereomicroscope microscope. (**B**) The same testis was observed for GFP fluorescence by confocal microscopy to observe GFP-expressing germ cells within the seminiferous tubules. Note lack of GFP-expressing germ cells in the 129-*Ter/Ter* testes. (**C**) H&E stained histological cross section of the PN1 testes from the 129-*Ter/Ter* mouse. *Arrow* indicates clusters of proliferating embryonal carcinoma (EC) cells. (**D**) Histological cross section, H&E stained, through testicular germ-cell tumor of adult 129-*Ter/Ter* mouse. The section shows randomly differentiated cell types of the teratoma. (**E**) Diagram illustrating mouse DND1 isoforms, DND1 α and DND1 β . DND1 α differs from DND1 β only at the first 45 and 33 amino acids of the N-terminus, respectively, as indicated by the length of *boxes* on the right. *Box* marked RRM indicates RNA recognition motif. *Asterisk* (*) indicates the position of the stop codon that inactivates DND1 in *Ter* mice because of the spontaneous mutation.