HYPOTHESIS

Germ cell determination and the developmental origin of germ cell tumors

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ABSTRACT

In each generation, the germline is tasked with producing somatic lineages that form the body, and segregating a population of cells for gametogenesis. During animal development, when do cells of the germline irreversibly commit to producing gametes? Integrating findings from diverse species, we conclude that the final commitment of the germline to gametogenesis – the process of germ cell determination – occurs after primordial germ cells (PGCs) colonize the gonads. Combining this understanding with medical findings, we present a model whereby germ cell tumors arise from cells that failed to undertake germ cell determination, regardless of their having colonized the gonads. We propose that the diversity of cell types present in these tumors reflects the broad developmental potential of migratory PGCs.

KEY WORDS: Determination, Embryo, Germ cell tumor, Germline, Potency, Primordial germ cell

Introduction

The germline is the cell lineage, from zygote to gamete, with the capacity or potential to contribute to the next generation (see Glossary, Table 1). Accordingly, a definitive test of whether a cell is of the germline or soma (see Glossary, Table 1) is genetic: all changes arising in germline cells can be inherited (assuming they do not impede gametogenesis), and no genetic change arising in the soma can be transmitted to offspring, an exclusion known as the 'Weismann barrier' (Fig. 1).

Within an animal, gametes trace their lineage back to primordial germ cells (PGCs), which arise during early development (reviewed by Strome and Updike, 2015; see Glossary, Table 1). After mammalian PGCs are induced, they migrate across the developing embryo to the nascent gonad, a journey widely conserved across metazoa (reviewed by Richardson and Lehmann, 2010).

When does the germline irreversibly commit to producing gametes and no other cell type – that is, when are germ cells determined? Here, we argue that this question is closely linked to the origin of germ cell tumors (GCTs; see Glossary, Table 1), which arise from the germline but are composed of diverse cell types. We compare germline development across diverse species to consider when and how determined (committed) germ cells are first distinguished from their germline precursors. We conclude that germ cell determination occurs late in development, after the body plan has been established and organogenesis begun, through a transition widely conserved among vertebrates. Synthesizing molecular and clinical findings, we

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hypothesize that mammalian GCTs reflect a failure of germ cell determination.

Germ cell determination occurs after PGC colonization of the gonads in vertebrates

Vertebrate PGCs retain the potential for both somatic and germ cell fates

Four lines of evidence support the view that migratory PGCs of mammals are not committed to gametogenesis. First, migratory PGCs express a network of pluripotency factors ('potency'; see Glossary, Table 1). In mice, this network includes factors characteristic of pre-implantation embryos and embryonic stem cells (ESCs), including the key transcription factors encoded by the genes *Nanog*, *Pou5f1* (encoding Oct4) and *Sox2* (Leitch and Smith, 2013; Nicholls et al., 2019). Like rodents, human PGCs express markers of the naïve pluripotent state. Unlike mice, human PGCs do not express *ESRRB* or *SOX2* (Perrett et al., 2008); instead *SOX17* functionally replaces *SOX2* by binding to the promoters of pluripotency factors and maintaining GCT cell lines in an undifferentiated state (Jostes et al., 2020). Second, PGCs can contribute to somatic lineages of the developing embryo. In mice, nascent PGCs expressing both *DPPA3* and *PRDM1* contribute to the allantois during normal development (Mikedis and Downs, 2012, 2017). Third, rodent PGCs give rise to pluripotent embryonic germ (EG) cell lines when cultured under conditions similar to those that facilitate derivation of ESCs, with no need for genetic transformation by pluripotency factors (Leitch et al., 2010, 2013; Matsui et al., 1992; Resnick et al., 1992). Human PGCs also produce transient EG-like cells when cultured (Kerr et al., 2008; Shamblott et al., 1998). Finally, PGCs can differentiate to produce teratomas (see Glossary, Table 1), a GCT that displays derivatives of all three (somatic) germ layers. Pioneering work by Leroy Stevens established that mouse PGCs can differentiate to produce spontaneous gonadal teratomas, or to experimentally-produced teratomas when PGCs are transplanted to ectopic locations such as the adult testis and spleen (Stevens, 1964). In humans, the germline can similarly produce GCTs (see below). Taken together, these many lines of evidence indicate that migratory PGCs of mammals are not committed to gametogenesis.

Although mouse PGCs have not been shown to contribute broadly to somatic lineages when transplanted into blastocysts, a study in pigs suggests this is possible in other mammals (Leitch et al., 2014; Mueller et al., 1999). The failure of mouse PGCs to produce chimeras may reflect a developmental incompatibility between PGCs and the blastocyst, as observed elsewhere when donor cells and the intended host environment are not well matched (Cohen et al., 2018).

This developmental potential of migratory PGCs in mammals is mirrored in non-mammalian vertebrates, irrespective of whether those PGCs arose via maternal inheritance or induction (see Glossary, Table 1; Fig. 1B). In frogs, transplantation of PGCs to ectopic sites reveals their capacity for differentiation to a somatic

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Table 1. Glossary

Term	Definition	Functional assay
Commitment	Multi-step process, whereby uncommitted cells progressively restrict their developmental potential, ultimately producing irreversibly committed cells.	See 'Specification' and 'Determination'.
Determination	Irreversible form of cell commitment.	Functional: will maintain its fate, regardless of its environment.
Germ cell	Cell of the germline, the potential of which is limited to producing gametes and no other cell type.	Functional: transplantation yields no somatic lineages, cannot acquire somatic fate in cell culture. Uniquely capable of meiotic cell cycle and gametogenesis.
Germ cell tumor (GCT)	Tumor arising from the germline. Reflecting the developmental potential of the initiating cell, GCTs may contain a broad array of cell lineages. Most commonly located in gonad (testis or ovary), GCTs may also arise along the route of PGC migration, such as the midline.	Traditionally defined by histological composition or epidemiology (e.g. age at presentation). Contemporary molecular findings indicate similar etiology, regardless of subtype (type I or type II; pediatric or adult; seminomatous or non-seminomatous; see Pierce et al., 2018 for further discussion). New biomarker approaches using miRNAs characteristic of pluripotent cells can detect malignant GCTs, irrespective of age of diagnosis, or subtype (with the exception of pure teratomas; Murray et al., 2016). Spermatocytic seminomas, dermatoid cysts and hydatidiform moles arise via different mechanisms and are not considered here.
Germline	Lineage of cells, from zygote through to gamete, that has the potential to give rise to cells of the next generation; they have properties of immortality.	Genetic: mutations occurring in cells of the germline may be passed to the next generation (presuming that mutation does not impair gametogenesis).
Primordial germ cell (PGC)	Cell of the germline set aside early in embryogenesis. Vertebrate PGCs, although precursors of gametes, are not yet determined; instead, they retain a broad developmental potential. PGCs may arise via maternally-derived cytoplasm or by induction (Figs 1 and 2).	Functional: PGCs are the only cells of an embryo with the potential for gametogenesis after gastrulation has occurred and somatic lineages have been allocated and determined. In many species, PGCs must migrate across the embryo to the gonad for gametogenesis.
PGC induction	Ancestral mode of germline development, occurring after zygotic genome activation (ZGA).	Expression: *Dnd1*, *Nanos* and pluripotency network (e.g. *Nanog*). Functional: undertake gametogenesis on gonadal transplantation.
Potency	The potential lineages a cell can produce. Totipotent cells have the potential to produce all lineages; pluripotent cells may give rise to all three germ layers and PGCs; unipotent cells have a singular potential.	Functional: see 'Specification' and 'Determination'.
Reprogramming	The process by which committed cells revert to pluripotency – a process that does not occur in normal development.	Functional: see 'Specification' and 'Determination'; achieved in somatic cells by forced expression of transcription factors to produce induced pluripotent stem cells (iPSCs).
Soma/somatic cells	Cells with no potential to give rise to gametes in the course of development; they are mortal.	Genetic: mutations occurring in somatic cells cannot be passed to the next generation.
Specification	Reversible form of cell commitment.	Functional: will develop autonomously along a developmental trajectory after isolation from the embryo and culture in 'neutral' conditions. May develop according to an ectopic niche if transplanted, or adopt a new fate in response to inductive signals.
Teratoma	GCT arising from pluripotent cells that have differentiated to produce all three germ layers.	Histology: derivatives of all three germ layers (ectoderm, endoderm and mesoderm).

cell fate according to the recipient niche (Wylie et al., 1985). Similarly, frog PGCs deficient in Nanos1 and located in the ectoderm are unable to evade the inductive influences of this niche, and activate ectoderm markers not usually expressed in the germline (Lai et al., 2012). In salamanders, nascent PGCs can be induced to express lineage-defining markers of somatic cells *ex vivo* (Chatfield et al., 2014). In zebrafish, PGCs deficient in the chemokine receptor Cxcr4 fail to respond to gonad-derived cues and instead migrate to ectopic locations throughout the developing embryo (Gross-Thebing et al., 2017). When these PGCs are also deficient for Dnd1, they differentiate to a somatic fate according to their new niche, indicating that Dnd1 functions to hold the somatic potential of migratory PGCs in check as they encounter ectopic niches (Gross-Thebing et al., 2017). Further, this raises the possibility that wild-type PGCs contribute to somatic lineages in the zebrafish, albeit at low frequency. Collectively, observations from a diverse array of mammalian and non-mammalian vertebrates demonstrate the broad potential of migratory PGCs for non-gametogenic fates –

including GCT formation – regardless of whether those PGCs arose via induction or maternal inheritance.

Anne McLaren defined germ cells (see Glossary, Table 1) as 'those cells, all of whose surviving descendants will become sperm and eggs' (McLaren, 2003). Thus, germ cells are distinguished from their immediate precursors when they lose the capacity for somatic fate(s). By this definition, vertebrate PGCs are not yet germ cells, as they retain the potential for non-gametogenic fates.

Evidence in mammals that germ cells are determined after PGCs colonize the gonads

Experimental studies of mammalian PGCs point to a commitment (see Glossary, Table 1) occurring after gonadal colonization. Using transplantation assays, Leroy Stevens discovered that germline potential is restricted within a few days of PGCs completing their migration across the embryo, between embryonic day (E) 12.5 and E14.5 in mice (Stevens, 1964, 1966, 1967). Consistent with this, the capacity for the derivation of pluripotent EG cells is restricted
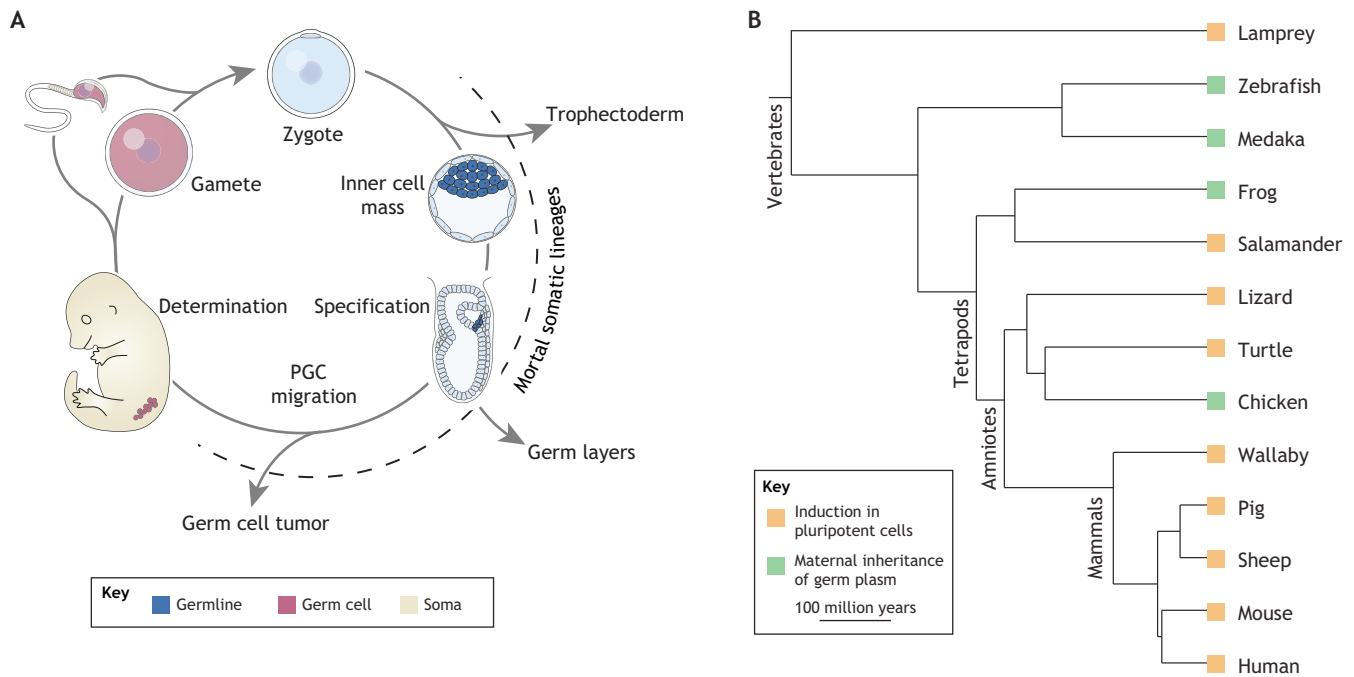
Fig. 1. The cycle of the germline varies widely among vertebrates. (A) The germline comprises all cells with the potential to produce cells of the next generation. Upon fertilization, the totipotent zygote has the capacity to give rise to all cell lineages, including gametes. As mammalian development proceeds, extra-embryonic and somatic lineages transit the figurative 'Weismann barrier' (dotted line), exiting the germline to adopt a mortal fate. This barrier can be overcome experimentally, e.g. in induced pluripotent stem cells, but not in normal development. Around gastrulation, a small population of primordial germ cells (PGCs) are induced, preserving the germline without irreversibly committing to gametogenesis. These cells migrate across the developing embryo and into the gonads. Upon PGC colonization of the gonads, a set of widely conserved germ cell factors are activated, leading to repression of the pluripotency network, and to germ cell determination. These committed germ cells (red) undertake sexual differentiation and gametogenesis, and then cycle through fertilization to re-establish totipotency and continue the germline lifecycle in a new individual. Failure of newly gonadal cells to undertake determination, or cell death, may instead yield gonadal germ cell tumors (Fig. 3). (B) The common ancestor of all vertebrates segregated its germline by induction of a germ cell program in pluripotent cells (orange). On at least three occasions during vertebrate evolution, this inductive mechanism was augmented by evolving maternally inherited 'germ plasm' (green), a polarized cytoplasm that causes recipient cells to become segregated germline very early in embryogenesis. Phylogenetic relationships from Hedges et al. (2015).

shortly after PGC colonization of the gonads in mice (Labosky et al., 1994; Matsui and Tokitake, 2009), as is the derivation of transient EG-like cells from human embryos (Kerr et al., 2008). Although long-term culture of spermatogonia can yield pluripotent cell lines, these arise exceptionally rarely and, unlike PGCs, transplantation of spermatogonia does not yield teratomas (Kanatsu-Shinohara et al., 2004; Takashima and Shinohara, 2018).

Revisiting the writings of earlier scholars, Jonathan Slack described two levels or states of commitment: specification and determination (Slack, 1991; see Glossary, Table 1). These two commitment states can be distinguished experimentally by grafting tissues and then examining whether cells develop according to the new niche, or according to their original position. By this definition, migratory PGCs of vertebrates may be specified, but their potential is not yet irreversibly restricted. By contrast, if a transplanted cell will only differentiate according to its original position, it is said to be determined, as its developmental potential is now fixed. Accordingly, germ cell determination occurs after PGC colonization in mice.

Analyzing the molecular profile of migratory and gonadal PGCs further reinforces the conclusion that germ cell determination occurs after gonad colonization. We recently examined the transcriptional changes that accompany PGC colonization of the gonads in humans and mice (Nicholls et al., 2019). We determined that genes expressed by migratory PGCs (such as *Dnd1*, *Nanos3*, *Prdm1* and *Tfap2c*) are expressed more broadly in somatic lineages in embryos and adults (i.e. their expression is not limited to germ cells or to the germline). In contrast, we identified a set of genes, first activated after PGC

colonization, that are exclusively or predominantly expressed by vertebrate germ cells; these include *Dazl*, *Ddx4*, *Mael* and *Tdrd12* (Table 2). These genes encode RNA-binding proteins that function in the maternally inherited germ plasm of non-mammalian vertebrates (including fish, frogs and chicken) and some invertebrates (Table 2) (Ewen-Campen et al., 2010). Thus, mammalian germ cell determination coincides with the induction of a set of widely conserved germ-cell factors. One such factor, *Dazl*, is first activated shortly after PGCs colonize the gonads in mice and humans, and is required for germ cell determination (Nicholls et al., 2019). When the *Dazl* gene is ablated, mouse PGCs arrive at the nascent gonad but continue to express pluripotency factors, including NANOG, SOX2 and LIN28 (Chen et al., 2014; Gill et al., 2011). The *Dazl*-deficient mouse germline also retains the capacity to give rise to pluripotent EG cells until at least E15.5, long after wild-type littermates lose this capacity, and almost 10 days after gastrulation (Nicholls et al., 2019). On most inbred genetic backgrounds, the *Dazl*-deficient germline fails to initiate sexual differentiation or gametogenesis and instead undertakes cell death, with few if any germline cells observed in the gonads of either sex by birth. In the 129-strain of mice (Box 1), some *Dazl*-deficient cells form testicular or ovarian teratomas, comprising cells from each of the three (somatic) germ layers. In pigs, an outgroup to rodents and primates (Fig. 1B), *DAZL* deficiency similarly leads to teratomas in ovaries and germline loss in testes (Nicholls et al., 2019). These data demonstrate that *Dazl* is necessary for germ cell determination, regardless of whether germline development occurs through induction or by maternal inheritance. Although expression of

Table 2. A widely conserved transition occurring at PGC colonization of the gonads

Organism (mode of segregation)	Expression of core pluripotency factors in PGCs: *Nanog*, PouV, SoxB	Expression of germ cell factors: *Dazl*, *Ddx4*	Germ cell determination
Mammal			
Mouse (induction)	*Nanog*, *Pou5f1* and *Sox2* expressed in migratory PGCs; extinguished after gonadal colonization.	First expressed after gonadal colonization; induced by somatic gonad.	Occurs after PGC colonization; dependent on *Dazl*.
Human (induction)	*NANOG* and *POU5F1* expressed in migratory PGCs; extinguished after gonadal colonization.	First expressed after gonadal colonization.	Occurs after PGC colonization.
Pig (induction)	*NANOG* and *POU5F1* expressed in PGCs until gonadal colonization (Kobayashi et al., 2017).	DAZL first expressed after gonadal colonization (Zhu et al., 2021).	Dependent on *DAZL* (Nicholls et al., 2019).
Wallaby (induction)	*POU5F1* expressed in PGCs until gonadal colonization (Frankenberg et al., 2010).	DDX4 first expressed after gonadal colonization (Hickford et al., 2011).	Unknown.
Diapsid			
Chicken (maternal inheritance)	*NANOG*, *POU5F3* and both *SOX2* and *SOX3* expressed by PGCs and early gonadal germline (Cañón et al., 2006; Jean et al., 2015).	*DAZL* and *DDX4* highly expressed after gonadal colonization (Jean et al., 2015).	Unknown.
Turtle (induction)	Unknown.	*Dazl* and *Ddx4* transcripts are detected in migratory PGCs; DDX4 protein first detected around the time PGCs colonize gonad (Bachvarova et al., 2009).	Unknown.
Amphibian			
Salamander (induction)	PouV and *Nanog* expressed in PGCs, and extinguished by the time PGCs are enveloped within gonad (Bachvarova et al., 2004; Dixon et al., 2010).	*Dazl* and *Ddx4* first expressed by the time PGCs arrive at gonad (Bachvarova et al., 2004; Johnson et al., 2001).	PGCs are uncommitted (Chatfield et al., 2014); when or how determination occurs is not known.
Frog (maternal inheritance)	PouV orthologs expressed throughout animal pole, with *pou5f3.1* limited to PGCs after gastrulation (Venkatarama et al., 2010).	*dazl* and *ddx4* are maternally supplied to PGCs. Maternal *dazl* not detected in PGCs between stages 26-31, with zygotic expression first occurring after colonization of gonads (Houston and King, 2000; Houston et al., 1998).	PGCs are uncommitted (Wylie et al., 1985); when or how determination occurs is not known.
Fish			
Zebrafish (maternal inheritance)	*nanog*, *pou5f3* and SoxB family expressed in migratory PGCs (Gross-Thebing et al., 2017; Sánchez-Sánchez et al., 2010); extinguished after gonadal colonization (Zhang et al., 2019).	*dazl* and *ddx4* are maternally supplied to PGCs; maternal *dazl* is not detected in PGCs at 30 h, with zygotic expression first occurring after colonization of the gonads; *dazl*-deficient PGCs do not initiate sexual differentiation or meiosis (Bertho et al., 2021; Nishimura et al., 2018).	PGCs are uncommitted (Gross-Thebing et al., 2017); when or how determination occurs is not known.

Time of last common ancestor with humans: mouse, 89 million years ago (MYA); pig, 94 MYA; wallaby, 160 MYA; diapsid, 318 MYA; amphibian, 352 MYA; fish, 433 MYA. Also see Fig. 2.

DAZL results in the downregulation of pluripotency factors in ESCs *in vitro* (Jung et al., 2017; Xu et al., 2013), it remains to be tested whether precocious expression of *Dazl* is sufficient for germ cell determination *in vivo*, before gonadal colonization.

In contrast to *Dazl*, no other widely conserved factors activated on PGC colonization of the embryonic gonads are necessary for germ cell determination. In the genetic absence of *Ddx4*, *Mael* or *Tdrd12*, PGCs initiate gametogenesis following arrival at the gonad, and females are fertile (Malki et al., 2014; Pandey et al., 2013; Tanaka et al., 2000). In the absence of *Dazl*, each of these other genes is induced after PGC colonization and, despite their expression, the germline retains the capacity for EG cell derivation and teratoma formation (Nicholls et al., 2019). Genetic ablation of *Ddx4*, a constituent of germ plasm in both vertebrates and invertebrates, does not increase the incidence of teratomas in the 129-strain of mice (Nicholls et al., 2019; Raz, 2000). Similarly, deletion of *Gcna*, another factor induced after PGCs colonize the gonad and present in the germ plasm of several model vertebrates and invertebrates, does not enhance teratoma incidence in mice (Carmell et al., 2016; Nicholls et al., 2019). Mechanistically, *Dazl*

functions to increase translational efficiency (Li et al., 2019; Mikedis et al., 2020), unlike other conserved germline factors, which typically repress gene expression (Seydoux and Braun, 2006). Thus, *Dazl* is unique among conserved germ cell factors: it is the first factor shown to be necessary for germ cell determination in vertebrates, regardless of whether the germline is segregated by maternal inheritance or by induction.

Together, this molecular and functional evidence supports a final restriction occurring after PGC colonization of the nascent gonad in mammals. It is likely that germ cells are the last lineage irreversibly committed in mammals, after organogenesis has commenced, almost 1 week after gastrulation in mice (Johnson and Alberio, 2015).

The transition occurring at PGC colonization is widely conserved among vertebrates

Comparing germ cell determination in mammals with observations in other vertebrates reveals a similar progression occurring around the time that PGCs colonize the gonads. As in eutherian mammals, migratory PGCs of other vertebrates are developmentally

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Box 1. Historical significance of the 129-strain of mice for biology and medicine
The mouse has long been a key model organism and surrogate for human development. In 1954, Leroy Stevens observed that 1% of juvenile males of the 129-strain of mice developed spontaneous testicular teratomas resembling those in humans (Stevens and Little, 1954). Stevens went on to establish the 129-strain – the only mammal to develop GCTs at an experimentally useful frequency – as a model for spontaneous teratomas, and for experimentally induced teratomas from transplanted cells (Stevens, 1964). The molecular genetic basis of the 129-strain's propensity to teratoma formation remains unknown.

Stevens' findings enabled discoveries that transformed biomedical research. For example, the first demonstration of the pluripotency of a single cell, the stem cell origin of cancer and the first culture of pluripotent cells lines were all undertaken with cells derived from testicular teratomas of the 129-strain of mice (Kahan and Ephrussi, 1970; Kleinsmith and Pierce, 1964). These advances ultimately led to the first derivation of ESCs and production of chimeric mice from cultured pluripotent cells – each contributing to the technological breakthrough of mice genetically modified through homologous recombination (Evans and Kaufman, 1981; Martin, 1981; Thomas and Capecchi, 1987). Accordingly, much of modern biology and medicine has emerged via the 129-strain's unique propensity to develop spontaneous teratomas and to give rise to pluripotent cell lines.

uncommitted and express transcription factors found in pluripotent cells, most notably Nanog, and the PouV and SoxB families (Table 2). These transcription factors are crucial *in vivo* for establishing pluripotency at the maternal-to-zygotic transition, and they are sufficient *in vitro* to reprogram mammalian somatic cells to pluripotency (Boyer et al., 2005; Lee et al., 2013; Takahashi and Yamanaka, 2006). In many vertebrates, this network of pluripotency factors continues to be expressed throughout PGC migration, being extinguished only after gonadal colonization (Table 2).

A second common feature of this germline transition is the induction of germ cell factors after gonadal arrival (Fig. 2). For example, in the Tammar wallaby, migratory PGCs continue to express *POU5F1* until their colonization of the gonad (Frankenberg et al., 2010). After gonadal arrival, these cells first express *DDX4*, just as in human and mouse embryos (Hickford et al., 2011). A similar pattern of gene expression is observed in birds and turtles. On gonadal colonization, both *DAZL* and *DDX4* are highly expressed in the chicken germline (Jean et al., 2015), as also occurs in turtles, where DDX4 protein is first detected (Bachvarova et al., 2009). In salamanders, where PGCs remain developmentally uncommitted, expression of PouV genes and *Nanog* is extinguished by the time PGCs are enveloped within the gonad (Bachvarova et al., 2004; Dixon et al., 2010). Like mammals, the salamander germline first expresses orthologs of *Dazl* and *Ddx4* as PGCs arrive at the future gonad (Bachvarova et al., 2004; Johnson et al., 2001). This developmental transition includes the period when salamander PGCs commit irreversibly (Chatfield et al., 2014). The simplest interpretation of these findings is that the transition at PGC colonization of the gonad occurred in the common ancestor of all tetrapods, and that this functioned to commit the germline to gametogenesis.

This transition also occurs in fish, where expression of *nanog*, *pou5f3* and *sox3* declines at PGC colonization, and zygotic expression of *dazl* first occurs after gonadal colonization (Bertho et al., 2021; Zhang et al., 2019). Although *dazl* is required for PGC formation (Hashimoto et al., 2004; Li et al., 2016), when medaka or zebrafish zygotes genetically deficient in *dazl* receive maternally supplied RNA, the resulting PGCs complete their migration to the gonad (Bertho et al., 2021; Nishimura et al., 2018). Once in the

gonad, genetically *dazl*-deficient cells are unable to initiate sexual differentiation or meiosis. These observations, together with genetic studies in *dnd1*-deficient zebrafish (Gross-Thebing et al., 2017), indicate that fish PGCs remain developmentally uncommitted through their migration, and that zygotic expression of *dazl* and other conserved germ plasm factors – after gonadal colonization – is required for the initiation of gametogenesis. Taken together with analogous findings in *Dazl*-deficient mice, these observations suggest that germ cell determination in fish occurs late in development, after PGC colonization of the gonad, and after induction of *dazl*.

In addition to these transcriptional changes, expression of factors required for piRNA biogenesis is also induced shortly after PGC arrival at the gonads. In mice, salamanders and fish, this causes a reorganization of RNA-rich cytoplasmic bodies known as germ granules (Aravin et al., 2009; Eddy, 1975; Ikenishi and Nieuwkoop, 1978; Redl et al., 2021; Voronina et al., 2011).

These comparative findings in diverse vertebrates provide evidence of a widely conserved transition occurring after PGC colonization of the nascent gonad (Table 2; Fig. 2). In many vertebrates, migratory PGCs express a network of pluripotency factors and remain developmentally uncommitted. Upon gonadal colonization, the germline initiates expression of a germ cell-defining program, characterized by the expression of two widely-conserved RNA-binding proteins, *Dazl* and *Ddx4*, and the re-organization of germ granules. This sequence of events, broadly conserved among model vertebrates, occurs regardless of whether germline and soma are initially segregated by induction or by maternal inheritance.

Germ cell tumors represent a failure of germ cell determination

Having examined germ cell commitment among diverse model vertebrates, we now explore how these comparative insights contribute to a revised understanding of the developmental origin of GCTs. We present a model whereby GCTs arise from germline cells that, having failed to make this final commitment after arrival at the gonad, retain a broad developmental potential. In humans, we hypothesize that this potential manifests as differentiation to the diverse histologies and cell types that are characteristic of GCTs.

An alternative model states that the first step toward tumorigenesis is the aberrant 'reprogramming' (see Glossary, Table 1) of germ cells to pluripotency (Oosterhuis and Looijenga, 2019). This alternative model is grounded in the view that mammalian PGCs are unipotent – built on the observation that mouse PGCs do not produce chimeras when injected into blastocysts (Leitch et al., 2014).

In the following sections, we examine how a failure of germ cell determination accounts for the full range of clinical, genetic and epidemiological observations regarding GCT pathogenesis, without the conceptual complexities of the reprogramming model.

Historical and clinical observations of GCTs

The pathologist Rudolf Virchow first recognized that testicular tumors contain a panoply of cell types and lineages not usually found in close proximity, and he named these tumors 'teratomas' (Virchow, 1863). On the basis of this cellular complexity, Virchow postulated that teratomas arise from 'indifferent elements' that developed in all possible directions – a concept of cell potential now termed pluripotency. Spontaneous teratomas arise from the germline and are observed in a variety of mammals, most frequently in the gonad (either testis or ovary) or occasionally along the migratory route of PGCs, such as in the adrenal glands or the midline (Anderson and
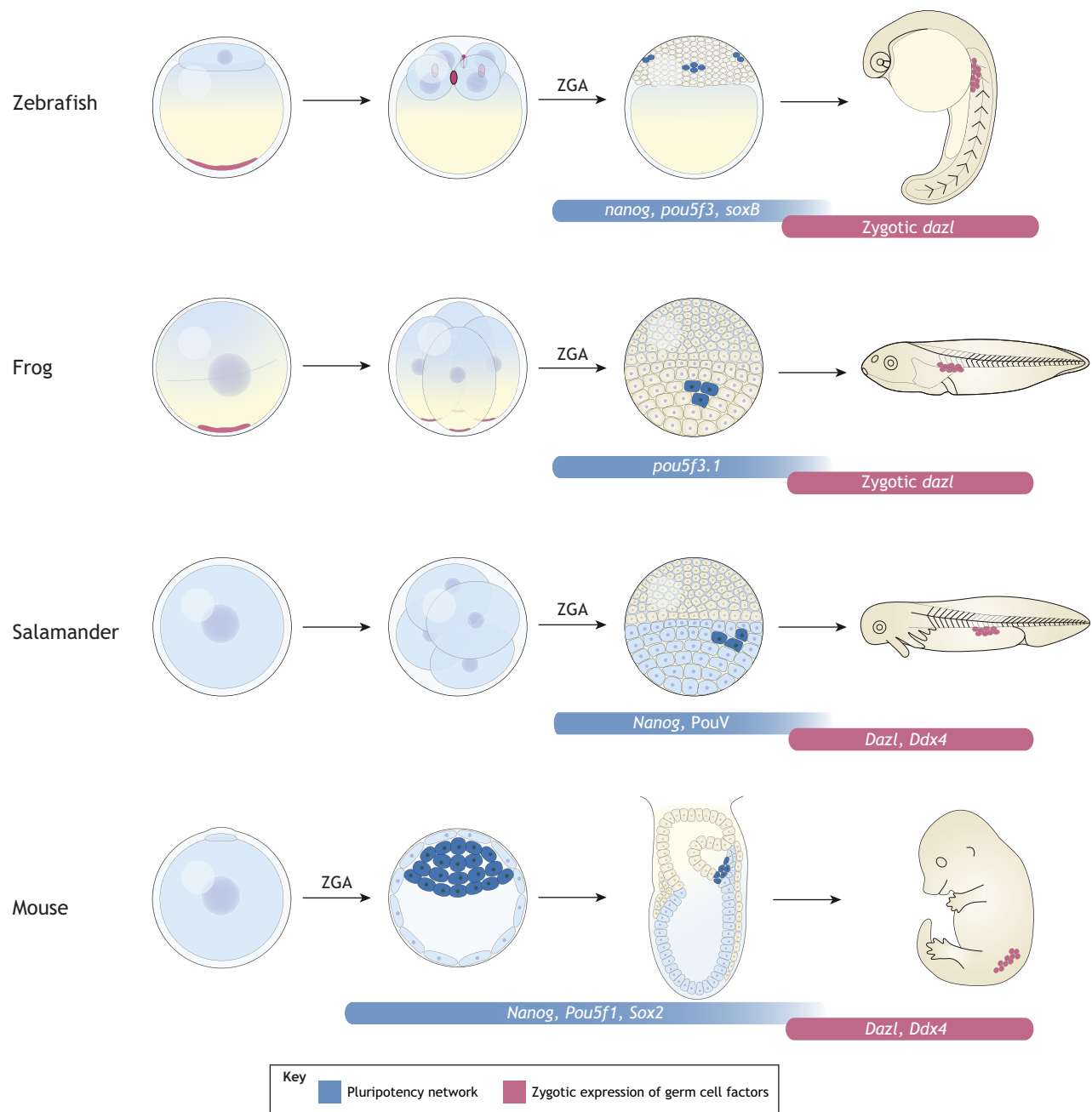
Fig. 2. A comparative view of germline commitment among vertebrates. Among four model vertebrates, the initial segregation of the germline (light blue) and somatic lineages (brown) is highly divergent, occurring early in the development of zebrafish and frogs through the action of maternally deposited 'germ plasm', but much later in the development of salamanders and mice, through induction of a zygotic program. Regardless of which mode is employed, the resulting germline migrates through the developing embryo and into the nascent gonads where it later undertakes gametogenesis. Throughout this migration, the PGCs of vertebrates remain developmentally uncommitted to solely producing gametes and no other cell type. This potential is indicated molecularly by an epigenetic and transcriptional profile resembling other pluripotent cell types, and by the differentiation to a somatic cell fate, regardless of whether this is realized in normal development. Upon colonization of the gonads, the germline of model vertebrates undertakes a transition, activating the zygotic expression of widely conserved germ cell factors (red) and subsequently repressing the pluripotency network (dark blue; expression of *Nanog*, PouV or SoxB family members). This transition, broadly conserved among vertebrates, is necessary for the determination of germ cells in mammals. ZGA, zygotic genome activation. Also see Table 2.

Johnson, 1988; Bishop, 1978; Steiner and Bengston, 1951; Stevens and Little, 1954; Willis, 1938). Spontaneous teratomas also arise in birds, providing further evidence that the tumor-forming potential of the germline is not unique to mammals (Ford et al., 2006; Sheather, 1911). Reflecting an embryonic origin, human GCTs are most prevalent in neonates, pre-pubertal children and young men. Teratomas, together with yolk sac tumors, are commonly referred to as type I germ cell tumors (GCTs), non-seminomatous GCTs

(NSGCTs), or pediatric GCTs (Oosterhuis and Looijenga, 2005; Pierce et al., 2018). Type II GCTs are histologically distinct (Box 2), being composed of either a pure seminoma or a mix of cell types comprising both seminoma and non-seminoma, such as choriocarcinoma, embryonal carcinoma, teratoma and yolk sac tumor. Irrespective of their presentation, genetic and epidemiological observations indicate that type I and type II GCTs share a common etiology (see below).

Box 2. Identification of germ cell neoplasia *in situ* as precursors of GCTs

Through a prospective clinical study, Niels Skakkebaek identified abnormal cells in two men who subsequently developed GCTs (Skakkebaek, 1972a,b). This observation raised the possibility that GCTs develop from an abnormal or undifferentiated precursor present in the testis before malignant transformation, termed germ cell neoplasia *in situ* (GCNIS; formerly, carcinoma *in situ*, CIS). It is now well established that type II GCTs can arise from GCNIS cells that remain dormant for many years, with epigenetic and transcriptional profiles similar to those of migratory PGCs, or of PGCs shortly after gonadal colonization (Sonne et al., 2009). Given these molecular similarities of GCTs to the embryonic germline, and their predominance in the young, one long-standing model posits that GCTs arise from germline cells blocked in some aspect of their development *in utero* (Rajpert De-Meyts and Hoei-Hansen, 2007; Rajpert-De Meyts et al., 1998).

Strong heritability reflects the embryonic origin of GCTs

GCTs are among the most heritable of all tumors, underscoring the importance of genetic contributions to GCT susceptibility. Sons or brothers of men with a GCT are at a two- and fourfold increased lifetime risk, respectively, a much greater degree of heritability than for other common cancers (Goldgar et al., 1994; Kharazmi et al., 2015). In men with two first-degree relatives who develop a GCT, the increase in lifetime risk is even greater, being 10- to 26-fold higher than in the general population (Kharazmi et al., 2015). Epidemiological studies find that the incidence of GCTs among US men of European ancestry is five times that among US men of African ancestry (Ghazarian et al., 2015). Despite clear heritability, there is no association between GCT subtype among first-degree relatives (Ghazarian et al., 2015); indicating that GCTs share a common etiology, irrespective of histology. These findings are further reinforced by cases of bilateral GCTs with discordant histology, despite identical genetic and environmental exposures (Thomas et al., 2013).

Given the heritability of GCTs, it was initially anticipated that rare high-penetrance alleles would be found in families with multiple affected members (Leahy et al., 1995). To date, such alleles have not been identified, or shown to account for familial heritability (Litchfield et al., 2018). Instead, genome wide association studies (GWAS) have enabled the discovery of prevalent low-effect alleles that confer heritability (Table 3). Such is the success of GWAS in mapping heritability that meta-analyses have found 49 risk loci that collectively account for 37% of the father-to-son familial risk of GCTs, irrespective of GCT subtype (Litchfield et al., 2017; Wang et al., 2017).

Although GCTs show strong heritability (indicative of risk alleles present in all cells), they simultaneously exhibit a very low rate of *de novo* mutations occurring in the tumor. Whole-exome studies estimate a mutation rate of ∼0.5 nucleotides per Mb, comparable with other pediatric cancers, but orders of magnitude less than other adult cancers (Shen et al., 2018). Anecdotal evidence further supports a model of GCT pathogenesis that is not built on an accumulation of *de novo* mutations across the lifespan: unlike many cancers, the risk of developing a GCT decreases substantially after the age of 35 (Znaor et al., 2015).

Genetic studies implicate the pluripotency network in GCT heritability

Taken together, genetic evidence indicates that GCT initiation does not depend on common *de novo* mutations in the precursor cell. Instead, meta-analyses of GWAS implicate alleles in several biological networks, thereby illuminating the pathways underlying GCT development (Table 3). These analyses implicate pluripotency factors, such as PRDM14, TFCP2L1 and ZFP42, which function in

pluripotency (see Glossary, Table 1) of the germline (Loveday et al., 2018; Rezende et al., 2011; Ruark et al., 2013; Wang et al., 2017; Yamaji et al., 2013; Ye et al., 2013). Additional risk alleles are predicted to disrupt genomic binding sites for pluripotency related transcription factors, such as POU5F1 binding to the *SALL4* promoter (Litchfield et al., 2017). Robust association with the pluripotency network, which is expressed in PGCs until shortly after colonization of the gonad, points to migratory PGCs, or PGCs that have recently arrived at the gonad, as the cells of origin for human GCTs (Figs 2 and 3).

What role does the pluripotency network play in GCT pathogenesis? Studies in ESCs and induced pluripotent stem cells (iPSCs) find that core pluripotency factors maintain self-renewal by limiting differentiation (Boyer et al., 2005). Mechanistically, this includes the maintenance of a 'poised' epigenetic state at the promoters of certain genes, expression of which would otherwise direct lineage commitment to a somatic fate (Bernstein et al., 2006; Lesch et al., 2016). For example, Nanog is required to maintain a repressive H3K27me3 histone mark at many developmental regulators, thereby restraining cells from differentiating (Heurtier et al., 2019). These observations raise the possibility that the pluripotency network functions to shield PGCs from inductive cues encountered along their migratory route through the embryo, thereby preserving the germline. Consistent with this, deletion or knockdown of either *Nanog*, *Pou5f1*, *Sox2*, the miR-290-295 cluster in migratory PGCs, or of *Lin28* in embryoid body-derived PGC-like cells, results in a dramatic reduction in the number of cells arriving at the nascent gonad of mice (Campolo et al., 2013; Kehler et al., 2004; Medeiros et al., 2011; West et al., 2009; Yamaguchi et al., 2009; Zhang et al., 2018). The effect of deleting such factors on tumorigenesis has not yet been explored in mice of suitable genetic background (Box 1).

Programmed cell death curtails germ cell tumorigenesis in mice and humans

Apoptosis features prominently in germline development and GCT heritability (Table 3). In mice deficient for *Bax*, a crucial effector of apoptosis, ectopic PGCs are retained along the migratory route, indicating that misplaced PGCs usually undergo programmed cell death (Runyan et al., 2008; Stallock et al., 2003). Although the fate of ectopic PGCs has not been examined, investigators have suggested that a failure of apoptosis may account for the development of GCTs along the midline in young children (Oosterhuis and Looijenga, 2019). Upon their arrival at the nascent gonad, PGCs that do not undertake *Dazl*-dependent germ cell determination initiate apoptosis in mice (Gill et al., 2011), as is also observed in testes of *DAZL*-deficient pigs and sheep (McLean et al., 2021; Nicholls et al., 2019). In the 129-strain of mice, some *Dazl*-deficient germline cells survive, and unilateral testicular teratomas form in one-third of mice. Simultaneously deleting *Bax* results in bilateral tumors in all 129-strain males (Nicholls et al., 2019). Deletion of *Bax* enables the survival of cells with a delayed or arrested development, and it increases the incidence of teratomas in mice carrying the *Ter* mutation (Cook et al., 2009, 2011; Nguyen et al., 2020).

Although BAX is the principal effector of apoptosis in the mouse germline, the human germline also expresses a second effector, BAK1, which is implicated by GWAS in the pathogenesis of GCTs of several subtypes (Poynter et al., 2012; Rapley et al., 2009). Similarly, the receptor/ligand pair KIT and KIT ligand (KITLG) function to protect cells from apoptosis in several developmental contexts, including the mouse germline (Runyan et al., 2006). As with *BAK1*, polymorphisms in *KITLG* are implicated by GWAS in the heritability of GCTs, and activating mutations in *KIT* frequently

Table 3. Genetic evidence in humans and mice implicates common pathways in GCT pathogenesis

Pathway	Gene (function)	Evidence from humans	Evidence from mice
Pluripotency network	*PRDM14*	GWAS (Ruark et al., 2013).	Expressed by vertebrate PGCs (Table 2); deletion invariably reduces size of the migratory PGC pool in mice (Campolo et al., 2013; Kehler et al., 2004; Medeiros et al., 2011; West et al., 2009; Yamaguchi et al., 2009; Zhang et al., 2018).
	SALL4	GWAS (Litchfield et al., 2017).	
	TFCP2L1	GWAS (Loveday et al., 2018; Wang et al., 2017).	
	ZFP42	GWAS (Litchfield et al., 2017; Wang et al., 2017).	
	Transcription factor-binding sites	GWAS; genomic binding sites for transcription factors, including KLF4, NANOG, POU5F1 and SOX2 (Litchfield et al., 2017).	
	isochromosome 12p	Increased copy number of *DPPA3*, *GDF3* and *NANOG* associated with tumor progression (Atkin and Baker, 1983; Ezeh et al., 2005; Ottesen et al., 2003; Shen et al., 2018; Taylor-Weiner et al., 2016).	
Apoptosis or cell survival	*BAK1* (apoptotic effector)	GWAS (Poynter et al., 2012; Rapley et al., 2009).	*Bax* (apoptotic effector) deletion increases teratoma incidence in mice carrying *Dazl* deletion or *Ter* allele (Cook et al., 2009, 2011; Nicholls et al., 2019).
	CHEK2 (activator of TP53)	Somatic mutations in case-control study (AlDubayan et al., 2019).	*Chek2*: apoptotic checkpoint in germline (Bolcun-Filas et al., 2014).
	KITLG (Kit ligand)	GWAS (Kanetsky et al., 2009; Poynter et al., 2012; Rapley et al., 2009).	*Kitl* promotes PGC survival (Runyan et al., 2006); Steel (*Sl*) alleles increase teratoma incidence (Heaney et al., 2008; Stevens and Mackensen, 1961).
	KIT (Kit receptor)	Somatic mutations in seminoma (Litchfield et al., 2017; Wang et al., 2017).	*Kit*: white-spotted (*W*) allele precludes GCT development (Heaney et al., 2008).
	MAP2K1 (*MEK1*, kinase)	GWAS (Litchfield et al., 2017; Wang et al., 2017).	Deletion is embryonic lethal (Giroux et al., 1999).
	MDM2 (activated by AKT; negative regulator of TP53)	Focal amplifications in GCTs (Bagrodia et al., 2016; Loveday et al., 2020; Shen et al., 2018); inhibition with Nutlin induces apoptosis in human GCT cell lines (Li et al., 2010).	Deletion is embryonic lethal (Jones et al., 1995; Montes de Oca Luna et al., 1995).
	PMAIP1 (*NOXA*, target of TP53)	Mediates apoptotic sensitivity in human GCT cell lines (Grande et al., 2012; Gutekunst et al., 2013).	Deletion enables germline survival following γ-irradiation (Kerr et al., 2012).
	PIK3C (PIP2 kinase)	*PIK3CA*: somatic mutations (Loveday et al., 2020; Shen et al., 2018). *PIK3CD*: GWAS (Litchfield et al., 2017), somatic mutations in seminoma (Shen et al., 2018) and in cisplatin-resistant GCTs (Feldman et al., 2014).	*Pik3ca* deletion is embryonic lethal (Bi et al., 1999).
	PTEN (PIP3 phosphatase)	Somatic mutations (Loveday et al., 2020).	Deletion increases teratoma incidence (Kimura et al., 2003; Pierpont et al., 2017).
	TP53	Li-Fraumeni syndrome associated with GCTs (Hartley et al., 1989); mutation implicated in cisplatin-resistant GCTs (Bagrodia et al., 2016; Loveday et al., 2020).	Deletion increases teratoma incidence (Harvey et al., 1993).
Germ cell commitment and gonadal development	DAZ family	*DAZ*: case-control studies of *gr/gr* men find increased relative risk of GCT (Moreno-Mendoza et al., 2019; Nathanson et al., 2005). *DAZL*: GWAS (Ruark et al., 2013).	*Dazl* deletion increases teratoma incidence in mice and pigs (Nicholls et al., 2019).
	DMRT1	GWAS (Kanetsky et al., 2011; Turnbull et al., 2010).	Required to maintain Sertoli cell differentiation status (Lindeman et al., 2015; Matson et al., 2011). Conditional germline deletion increases teratoma incidence (Krentz et al., 2009, 2013).
	GATA4	GWAS (Litchfield et al., 2017).	Required for differentiation of the nascent gonad and *Dazl* expression in PGCs after colonization (Hu et al., 2013, 2015).
	Gonadal development	Epidemiology: disorders of sex-development are associated with increased GCT risk (Pleskacova et al., 2010). GWAS: Enrichment for sex-determination pathways (Koster et al., 2014).	Greater incidence of teratoma in testes than ovaries, irrespective of sex chromosome constitution (Nicholls et al., 2019).

GCT, germ cell tumor; GWAS, genome-wide association study.

accompany the transformation of precursor germ cell neoplasia *in situ* (GCNIS) cells into overt seminomas (Biermann et al., 2007; Kanetsky et al., 2009; Litchfield et al., 2015; Shen et al., 2018; Taylor-Weiner et al., 2016). Downstream of KIT signaling, the AKT and MAPK pathways are similarly implicated in the pathogenesis of GCTs in humans and mice (Kimura et al., 2003; Litchfield et al., 2017; Pierpont et al., 2017; Wang et al., 2017). These broadly consistent findings in mice and humans implicate an escape from apoptosis and the resulting survival of cells that, having failed to restrict their developmental potential, endure in the embryonic
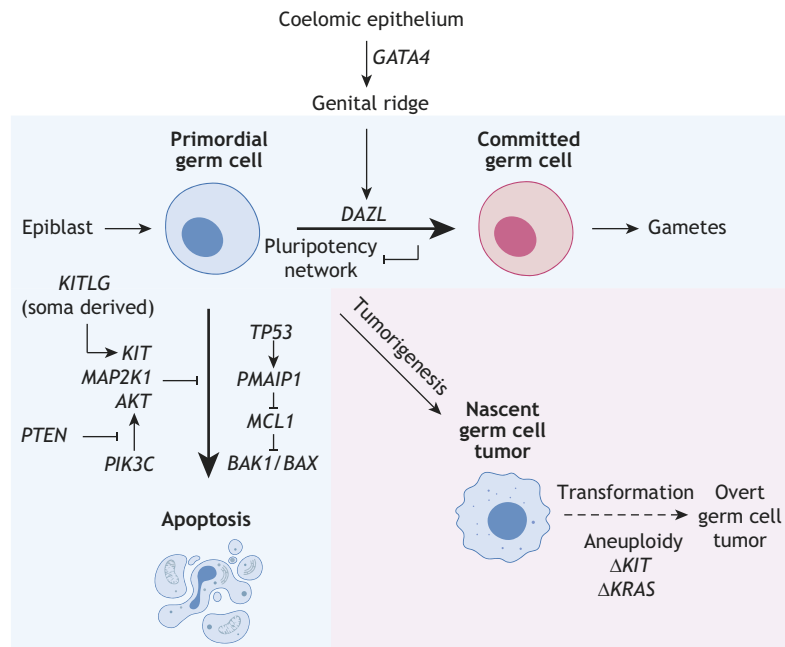
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Fig. 3. A model for germline development in mammals and for the origin of germ cell tumors. In normal mammalian development, primordial germ cells (PGCs) migrate through the embryo and into the nascent gonad, in which soma-derived signals induce germ cell commitment (determination), dependent on the RNA-binding protein DAZL. Once committed, these gonadal cells are competent to initiate gametogenesis. When this commitment process fails to occur appropriately, PGCs retain the expression of a network of pluripotency factors. To avert tumorigenesis, these gonadal PGCs usually undertake apoptosis, which likely occurs in a TP53-dependent manner through either BAK1 (human) or BAX (mouse). We hypothesize that intracellular signaling via KIT, MAP2K1 and AKT promotes cell survival, thereby enabling the survival of cells that would otherwise undertake apoptosis. When germ cell determination and apoptosis fail to occur, the gonad is endowed with a population of cells that retain the developmental potential of PGCs, which may undertake tumorigenesis. Subsequent transformation events produce overt germ cell tumors, characterized by chromosomal aneuploidy and recurrent mutations to *KIT* or *KRAS*. Each gene shown has been implicated in the heritability of GCTs in humans, and also in germline development or susceptibility to teratoma formation in mice. These include the differentiation of the gonad from the coelomic epithelium (*GATA4*), germ cell commitment (*DAZL*), the pluripotency network expressed by migratory and newly gonadal PGCs (*PRDM14*, *TFCP2L1*, *ZFP42*, *SALL4*, and genomic binding sites of KLF4, NANOG, POU5F1 and SOX2), and of cell survival pathways and apoptosis (*BAK1*, *KITLG*, *MAP2K1*, *PIK3CD* and *PTEN*).

gonad (or along the midline) and remain susceptible to tumorigenesis (Fig. 3).

Differentiation of the somatic gonad instructs germ cell commitment
As discussed earlier, germ cell determination restricts germline potential, precluding tumorigenesis. Genetic findings in humans implicate the differentiation of the gonadal soma and the DAZ family of genes in the pathogenesis of GCTs, regardless of histological subtype. As a result of transpositions and duplications, the human XY genome includes six members of the DAZ gene family: four copies of *DAZ* on the Y chromosome, and the single-copy autosomal genes *DAZL* and *BOLL* (Hughes et al., 2012; Saxena et al., 1996). *De novo* structural rearrangements of the Y chromosome regularly affect *DAZ* copy number and are a significant cause of spermatogenic failure (Rozen et al., 2012). One common Y-chromosome rearrangement, the *gr/gr* deletion, removes two copies of *DAZ* and is associated with an elevated GCT risk, particularly in men with a family history of GCTs (Moreno-Mendoza et al., 2019; Nathanson et al., 2005; Repping et al., 2003). Furthermore, the autosomal gene *DAZL* is directly implicated in the heritability of GCTs by GWAS (Ruark et al., 2013). Given genetic evidence of the role of *Dazl* in the shared ancestor of mice and pigs, and of the important role of the DAZ family in humans, the DAZ family likely functions in the restriction of germline potency in humans, precluding tumorigenesis (Fig. 3).

Further support for this model comes from evidence implicating the gonadal soma in inducing germ cell determination. In mice, PGCs arrive at the nascent gonad as it simultaneously differentiates in a

Gata4-dependent manner (Hu et al., 2013). The differentiation of the gonad is necessary for the subsequent induction of *Dazl* in the germline (as also occurs when PGC-like cells are cultured with a gonad), leading to germ cell determination and the initiation of gametogenesis (Hayashi et al., 2012; Hu et al., 2015; Nicholls et al., 2019). Several factors known to function in somatic cells of the nascent gonad, including DMRT1 and GATA4, have been implicated in GCT formation by GWAS (Koster et al., 2014; Litchfield et al., 2017; Turnbull et al., 2010). In mice, these transcription factors function to build and maintain the somatic lineages of the gonad (Hu et al., 2013; Lindeman et al., 2015; Matson et al., 2011). These genetic observations in humans support the model that the embryonic gonad exerts an instructive function in germ cell determination. Epidemiological evidence further implicates the developing gonad, as several syndromes associated with gonadal dysgenesis greatly elevate GCT risk (Pleskacova et al., 2010).

Somatic mutations promote malignancy but are not necessary for GCT formation
Consistent with an embryonic origin for GCTs, their cellular precursors generally lack *de novo* mutations. Nonetheless, certain characteristic genetic events drive or accompany the subsequent transformation of benign GCTs to malignancy (Fig. 3). Evidence suggests that a whole genome duplication – likely occurring in benign GCTs – is an early transformation event, followed by several rounds of chromosome arm loss yielding a complex chromosomal complement (Shen et al., 2018; Taylor-Weiner et al., 2016). Although the ploidy of

late-stage GCTs is variable, there is often retention (effectively amplification) of chromosome 12p, regardless of tumor histology or subtype (Atkin and Baker, 1983; Shen et al., 2018; Taylor-Weiner et al., 2016). The implicated region of chromosome 12p encompasses the proto-oncogene *KRAS* (a cluster of factors involved in control of pluripotency, including *NANOG*, *DPPA3* and *GDF3*; Ezeh et al., 2005) and the cell-cycle regulator *CCND2*. This characteristic amplification of chromosome 12p is not found in all cells of a GCT (or in all GCTs), suggesting that it is not required for tumorigenesis, but is frequently acquired during tumor progression (Ottesen et al., 2003).

Pure seminomas frequently exhibit mutations in either *KRAS* or *KIT* – the only two recurrent sites of mutations (Shen et al., 2018; Taylor-Weiner et al., 2016). Such mutations in *KIT* are seen in about half of pure seminomas but are rarely observed in non-seminoma or histologically heterogeneous GCTs (Shen et al., 2018). Thus, the initial development of GCTs from the embryonic germline does not require these characteristic genetic changes, which later promote transformation to a malignant phenotype (Fig. 3).

The 129-strain of mice recapitulates the pathogenesis of human GCTs

Despite the widespread use of the 129-strain of mice, the genetic basis for spontaneous gonadal teratomas in this strain is unknown (Heaney and Nadeau, 2008) (Box 1). Regardless, the 129-strain has long provided the most tractable model to interrogate the genetics of teratoma development. For example, 129-strain male mice carrying the *Steel* allele (*Sl^J*, a 650-kb deletion encompassing *Kitl*) develop teratomas at a rate seven times that of their wild-type littermates (Heaney et al., 2008; Stevens and Mackensen, 1961). In contrast, mice carrying two copies of *Sl^J*, or a deletion of *Kit* (the receptor of *Kitl*), are deficient in germ cells and do not develop teratomas (Stevens, 1967). These findings provided the first definitive evidence that teratomas arise from the germline and not from the gonadal soma. Leroy Stevens later isolated a spontaneous mutation in his colony of 129-strain mice, named the *Ter* allele, where one-third of mice heterozygous for the allele develop testicular teratomas (Stevens, 1973). Subsequent studies mapped *Ter* to a point-mutation in *Dnd1* on chromosome 18 (Asada et al., 1994; Sakurai et al., 1994; Youngren et al., 2005). Although the incidence of teratomas in mice homozygous for *Dnd1^Ter* is over 90% in a 129-strain background, teratomas do not form when this allele is transferred to the C57BL/6-strain, even when secondary modifiers are included (Cook et al., 2011; Noguchi et al., 1996). Deletion of many genes similarly modifies teratoma incidence in the 129-strain (such as *Dazl*, *Dmrt1*, *Nanos3* or *Tfap2c*), but each causes germline apoptosis and sterility on the C57BL/6-strain, and no teratomas arise (Krentz et al., 2009; Nicholls et al., 2019; Noguchi et al., 1996; Schemmer et al., 2013; Suzuki et al., 2008). Although teratomas develop with regularity in the 129-strain, these tumors contain undifferentiated embryonal carcinoma cells (ECCs, characteristic of mixed GCTs) at very low frequency (Schemmer et al., 2013). Conversely, mice deficient in *Pten*, with a second activating mutation in *Kras*, develop testicular GCTs with many ECCs (Pierpont et al., 2017). As in humans, these ECCs are sensitive to standard therapy and this model may facilitate the validation of novel agents.

Like human GCTs, spontaneously arising teratomas occur in juvenile mice, reflecting their origin from the embryonic germline (Dawson et al., 2018; Heaney et al., 2012; Pierpont et al., 2017). Further, the strong sex-bias towards males reflects the burden of human disease (Nicholls et al., 2019; Poynter et al., 2010). Despite these similarities, spontaneous GCTs in mice are teratomas or display a mixed GCT histology; no mouse model containing a seminoma

component has been described. Although yolk sac and trophoblast elements are rarely seen in mice *in vivo*, they can differentiate from teratoma-derived cell lines (Stevens, 1960). Given the failure to fully recapitulate a seminoma-like histology, the 129-strain has sometimes been dismissed as irrelevant to human GCTs, particularly those of post-pubertal men (Almstrup et al., 2006; Looijenga et al., 2014; Walt et al., 1993). Despite these differences, the underlying heritability of human GCTs, irrespective of histological subtype, converges with observations in mice (Table 3; Fig. 3). For example, *Kitl* deletions elevate the incidence of teratoma formation in mice, and GWAS has implicated *KITLG* in the pathogenesis of GCTs in men (Heaney et al., 2008; Kanetsky et al., 2009; Rapley et al., 2009). Similarly, human GWAS have implicated factors important for germ cell commitment, such as *DAZL* and *DMRT1*, the deletion of which dramatically increases teratoma incidence in mice (Krentz et al., 2009; Nicholls et al., 2019; Ruark et al., 2013; Turnbull et al., 2010). Likewise, apoptotic effectors *BAK1* and *Bax* have been implicated in human and mouse GCTs, respectively (Cook et al., 2009, 2011; Nicholls et al., 2019; Poynter et al., 2012; Rapley et al., 2009). Given rapid progress in understanding the heritability of human GCTs, genetic approaches can now be employed to better interrogate GCT development in mouse models. As such, the 129-strain of mice constitutes a valuable resource with which to interrogate the developmental origin and biology of human GCTs.

A failure of determination, rather than reprogramming, better explains the origin of GCTs

Complementary observations indicate that GCTs arise from a failure of development: the young age at first diagnosis; high familial heritability; the absence of driver mutations; transcriptional and epigenetic similarity to the embryonic germline; a developmental potential unique to the embryonic germline; and reduced risk with increasing age. Each finding bolsters the view that GCTs arise from the embryonic germline, shortly after gonadal colonization. Which model – a failure of germ cell determination on arrival at the gonad, or the 'reprogramming' of unipotent cells to pluripotency – better accounts for the cellular origins of these tumors?

Previous efforts to define the developmental origins of GCTs have pointed to the transcriptional profile or epigenetic state as evidence of an embryonic cell of origin (Oosterhuis and Looijenga, 2019; Sonne et al., 2009). Although GCTs express many core and naïve pluripotency factors (markers normally restricted to migratory PGCs of vertebrates), this expression cannot resolve the precise origin of GCTs; this could indicate that GCTs arise from cells that failed to extinguish such factors following gonadal colonization, or alternatively, from differentiated cells that reacquired their expression. Like gene expression, genomic imprinting status provides clues (Schneider et al., 2001), but interpretation is complicated by ongoing expression of writers and erasers of DNA methylation (DNA methyltransferases and ten-eleven translocation enzymes, respectively) in the developing germline. Thus, it has remained unresolved whether GCTs arise from PGCs that failed to undertake determination on arrival at the gonad, or from more advanced cells that have reprogrammed to pluripotency.

The reprogramming model states that, as the initiating event of tumorigenesis, PGCs must first aberrantly reprogram to pluripotency (Oosterhuis and Looijenga, 2019). This model is grounded in the view that mouse PGCs are unipotent. Evidence to the contrary, such as derivation of pluripotent cell lines and teratomas, has been interpreted as confirming the reprogramming (de-differentiation) of PGCs to a broader potential not normally held by these cells (Leitch and Smith, 2013). Although a large network of factors sufficient to

reprogram somatic cells to pluripotency – exemplified by iPSCs, which revert across the 'Weismann barrier' – have been interrogated at great depth, most (if not all) are expressed in mammalian PGCs and other model vertebrates. Thus, any reprogramming would be elicited by factors that are normally expressed by PGCs. To account for the inefficiency of reprogramming, it has been argued that reduced apoptosis, such as occurs in *Bax*-deficient mice, serves to increase the number of PGCs available for reprogramming (Oosterhuis and Looijenga, 2019). Yet deletion of *Bax* alone does not produce any obvious increase in the incidence of teratomas in otherwise susceptible mice (Nicholls et al., 2019). In mice additionally depleted of *Dazl* (that fail to undertake determination), the incidence of bilateral teratomas far outweighs any change in the number of PGCs. Conversely, the *Ter* mutation to *Dnd1* causes a large deficiency of PGCs arriving at the gonad, yet the incidence of teratomas in mice carrying this allele is remarkably high (Sakurai et al., 1995; Stevens, 1973). The reprogramming hypothesis would further require that *Dazl* is necessary to prevent the reprogramming of unipotent cells to pluripotency.

Integrating findings from diverse vertebrates, we advocate a simpler and more parsimonious model: that mammalian PGCs are not committed to gametogenesis. In our model, newly gonadal PGCs that fail to undertake germ cell determination remain pluripotent and are susceptible to tumor formation. The broad array of cell lineages and histologies found in human GCTs (and the absence of driver mutations at tumor initiation) reflect the broad potential of migratory PGCs across Vertebrata. We propose that the survival of such pluripotent cells is a first step of tumorigenesis (Fig. 3), before transformation or differentiation to an overtly tumorigenic phenotype (Dawson et al., 2018).

The development of GCTs, from uncommitted cells of the germline, before sexual differentiation, may also account for the common pathogenesis of ovarian and testicular GCTs (Box 3). We reason that this model better accounts for the broader range of evidence with fewer assumptions, consistent with the demands of Occam's razor. Although the precise origin of human GCNIS cells remains to be definitively established (see Box 2), observations in mice and other vertebrates provide the first genetic evidence for how germ cells are determined – a process that prevents tumorigenesis.

Conclusions

During the past decade, insights and resources from a range of model organisms have enabled a re-evaluation of germline development and the origin of GCTs. A recurring theme emerges: that human GCTs arise from a failure of conserved processes operating in the embryonic germline of mammals and non-mammalian vertebrates. Of importance, several processes that influence germ cell commitment in model organisms are also implicated in the pathogenesis of human GCTs. These processes include pathways that maintain the developmental potency of the germline, apoptotic cell death and the soma-derived induction of germ cell commitment (Table 3).

Current models assume that PGCs are unipotent germ cells, rationalizing observations to the contrary as artifacts of aberrant reprogramming of PGCs to pluripotency. The arguments for the unipotency of PGCs are belied by the demonstrable potential of migratory PGCs in fish, frogs, salamanders, mice and humans. Furthermore, the derivation of EG cell lines – without genetic transformation – indicates that migratory PGCs of mice readily give rise to pluripotent cell lines. No reprogramming of PGCs before tumorigenesis is required if migratory stage-PGCs are developmentally uncommitted cells of the germline. This simplified model is consistent

Box 3. Similar pathogenesis of GCTs of the ovary or testis

GCTs of the human ovary share many characteristics with their testicular counterparts. Germinomas and non-germinomas (the ovarian equivalent of seminoma and non-seminoma, respectively) arise early in life, express a network of pluripotency factors, show polyploidy and additional copies of chromosome 12p, and frequently acquire mutations in *KIT* or *KRAS* (Hoei-Hansen et al., 2007; Malecki et al., 2012; Van Nieuwenhuysen et al., 2018). GCTs of the ovary are similarly chemosensitive, with survival rates matching those of testicular GCTs (Weinberg et al., 2011). Epidemiological studies have also shown that ovarian GCTs are more common in individuals of European rather than African ancestry (Smith et al., 2006). These many histological, molecular and epidemiological similarities between ovarian and testicular GCTs suggest these two pathologies may represent a single developmental disorder, irrespective of the gonadal environment or the sex chromosome constitution (Kraggerud et al., 2013; Teilum, 1944).

Given the remarkable similarities of ovarian and testicular GCTs (excluding those arising via parthenogenesis), it appears plausible that both arise from a common precursor cell. In mice, PGCs arrive at the nascent gonad before they undergo sex determination (Adams and McLaren, 2002). If these tumors share a common origin, it would likely occur in the brief window after PGCs arrive at the nascent gonads (the site of the majority of GCTs), but before their sexual differentiation. This developmental window includes the period when PGCs undertake germ cell determination, and a failure to undertake this commitment, in either sex, may later produce GCTs in mice (Nicholls et al., 2019).

Ovarian and testicular GCTs differ in two important aspects: the sex chromosome constitution of the cells (XX in female, XY in male), and the gonadal niche encountered by the newly colonized PGCs (ovary or testis). The development of spontaneous teratomas in mice of both sexes provides an opportunity to genetically dissect the influence of the sex chromosomes and of gonadal sex (Arnold and Chen, 2009). Using a sex-reversed model, the incidence of tumors is greater in testes, indicating that the testicular niche facilitates the development of gonadal GCTs, irrespective of sex chromosome constitution (Nicholls et al., 2019). Although the mechanism underlying these sex differences awaits further study, a plethora of growth factors produced by the developing testes may affect either the self-renewal or differentiation of the newly arrived germline (Bowles et al., 2010; Colvin et al., 2001; Miles et al., 2013; Spiller et al., 2012).

with other childhood cancers, such as pediatric brain tumors, for which an abundance of evidence suggests that they arise from cells blocked in a self-renewing progenitor-like state (Jessa et al., 2019).

Germ cell tumors remain a significant societal burden. Despite the stunning success of current therapy – with a five-year survival rate in excess of 95% (Stang et al., 2013) – 10,000 men will succumb to testis cancer each year (Global Burden of Disease Cancer Collaboration et al., 2017). Given the young age at diagnosis, GCTs are responsible for many years of healthy life lost. Further, the global incidence of GCTs has been rising steadily in recent decades, with the causes likely to be found in embryonic and fetal development (Skakkebæk et al., 2001; Znaor et al., 2015). The formulation of new therapeutic and diagnostic approaches, and an understanding of the dramatic increase in GCT incidence, depend upon a deeper knowledge of the origins of these tumors.

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