RBM ONLINE

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Commentary

Extragonadal primordial germ cells or placental progenitor cells?

Karen M Downs *

Department of Cell and Regenerative Biology, University of Wisconsin-Madison School of Medicine and Public Health, 1300 University Avenue, Madison, WI 53706, USA

A B S T R A C T

Primordial germ cells (PGC), the precursors of the gametes, are now claimed to segregate within the extra-embryonic tissues of three species of placental mammals. In this brief Commentary, I raise the question of whether the so-called PGC are not PGC at all, but rather, progenitor cells that build the fetal–placental interface in Placentalia.

Introduction

Biologists have long been preoccupied with the origin of the mammalian germ line. At present the general consensus, based on studies in the mouse model, is that its antecedents arise within the pluripotent epiblast, move to the extra-embryonic region where they segregate from the soma, and from there, primordial germ cells (PGC) re-enter the embryo to migrate to the gonads via the hindgut. This conclusion is based on over a century of description, beginning with morphology (Simkins, 1923), and followed by biochemical activity for alkaline phosphatase (AlkP) (Chiquoine, 1954; Witschi, 1948). Then, once molecular methods became available for sorting small cell populations, AlkP-positive cells were probed for genes 'uniquely' expressed at the embryonic–extra-embryonic interface (Ohinata et al., 2005; Saitou et al., 2002). At present, gene expression is the *status quo* by which PGC are identified and studied in the posterior region of Placentalia.

Despite current efforts directed toward establishing higher orders of gene expression within AlkP-positive cells (e.g. Sasaki et al., 2016), germ cell biologists have overlooked a fundamental principle of developmental biology: gene expression and cell lineage do not form an obligate ancestral relationship (Beddington, 1988). In the case of the germ line, not a single study has demonstrated that extra-embryonically 'segregating' PGC end up in the gonads (reviewed in Mikedis and Downs, 2014). A similar lament was published nearly a century ago when PGC were claimed to be recognizable by morphology (Simkins, 1923):

'Many investigators have considered the problem solved in favour of a morphological continuity of germ cells when certain cells, designated as primordial germ cells, have been followed from their place of origin over long distances of intervening structures to the site of the germ-gland fundament. Some workers have been so sure that these cells were the true fore-runners of the reproductive cells that they have not followed the history of them through the critical period of gonogenesis, although such study is essential for determining whether or not these cells actually become transformed into definitive ova and spermatozoa; it was assumed that they were, because of certain resemblances which they bore to the early oogonia and spermatogonia.'

In this brief Commentary, I point out that fate mapping presumptive PGC from the extra-embryonic region of the mammalian conceptus to the gonads remains elusive. Further, based on new insights into the properties of the posterior embryonic–extra-embryonic region, where PGC are thought to segregate from the soma, I ask whether the so-called extra-embryonic antecedents to the germ line are not PGC at all, but rather progenitor cells that build the fetal–placental

* *E-mail address:* kdowns@wisc.edu.

https://doi.org/10.1016/j.rbmo.2017.09.013

1472-6483/

Please cite this article in press as: Karen M. Downs, Extragonadal primordial germ cells or placental progenitor cells?, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.09.013

interface. I conclude with current – and equally controversial – thoughts about where mammalian germ line progenitors might originate.

Origin of the germ line

Pluripotency is expected to be a major feature of PGC which, after maturation into the gametes and their subsequent union, recapitulate the entire organism. During cleavage stages, all blastomeres appear capable of contributing to the germ line (Tarkowski et al., 2010). Upon formation of the blastocyst, two major cell types appear: the inner cell mass (ICM) and the trophoblast ('trophectoderm' in mice). Trophectoderm does not contribute to tissues other than the placentae (Gardner et al., 1973; Papioannou, 1982; Tanaka et al., 1998), while individual cells of the ICM are pluripotent (Gardner and Lyon, 1971). At implantation, the ICM differentiates into the epiblast and primitive endoderm. Pluripotent epiblast cells contribute to soma, both embryonic and extra-embryonic, and to the germ line (Gardner et al., 1985). By contrast, primitive endoderm has not historically demonstrated such widespread potential (Gardner, 1972; Gardner and Rossant, 1979; Kunath et al., 2005). Thus, the germ line resides somewhere within the epiblast.

During gastrulation, the embryo becomes apparent as a result of formation of the primitive streak, or antero-posterior body axis (Corner, 1944); the embryo's associated extra-embryonic tissues, amnion, allantois, yolk sac and chorion, coalesce into two major placentae, the chorio-allantoic placenta and the chorio-vitelline (yolk sac) placenta, which collaborate to create a patterned and efficient vascular conduit from the fetus to its mother for exchange of nutrients, wastes and gases within the womb.

Epiblast-derived cells enter the posterior extra-embryonic space and there, segregate from the soma: yolk sac visceral endoderm in humans (Witschi, 1948), the base of the allantois in the mouse (Lawson and Hage, 1994; Ozdzenski, 1967), and the amnion in a non-human primate (Sasaki et al., 2016). Is there a pattern? Yes, it would seem so: major tissue components of placental tissues segregate PGC.

Why would the mammalian germ line come into being at such great distances from the eventual site of gonad formation? The most cited rationale is that pressures of determination and differentiation in the embryo during gastrulation threaten PGC potency that must be preserved (McLaren, 1992). As a result, germ cell antecedents take refuge in extra-embryonic tissues, segregating there and then re-entering the embryo when the coast is clear. However, not only is a formal demonstration of continuity between putative PGC and the gonads lacking, but this proposition is wholly embryo-centric: it fails to acknowledge that extra-embryonic tissues have properties, too. For example, by contrast with the epiblast, extra-embryonic tissues can sustain higher orders of ploidy (Tarkowski et al., 1977). Thus, the extra-embryonic environment is more tolerant than the embryonic one in accommodating cells that accumulate changes in chromosomal number. On that basis, an extra-embryonic site would hardly seem the place to invite formation of the future germ line, whose chromosomal integrity must be upheld.

As far as McLaren's claim that gastrulation is confined to the embryo is concerned, this long-held belief has recently been challenged. Gastrulation is defined by the activity of the primitive streak (Snell and Stevens, 1966). Contrary to prevailing notions (Downs, 2009), the mouse primitive streak is not limited to the embryo proper (Sobotta, 1911) but rather, extends into the allantois, where its posterior terminus expands into a dense cellular core, defined by Brachyury, and provisionally named the 'allantoic core domain' (ACD) (Downs et al., 2009). Fate mapping showed that this extra-embryonic ACD, like the embryonic primitive streak (Tam and Beddington, 1987), is a pluripotent progenitor cell pool that contributes to derivatives of all three primary germ layers at the fetal–placental interface, both embryonic and extra-embryonic (Mikedis and Downs, 2012). Thus, gastrulation encompasses the extra-embryonic region, too.

The discovery of the ACD within the base of the allantois represented a paradigm shift in understanding the biology of Mammalia, because a primitive streak that extends into the allantois possesses not only progenitor cells that build this vital organ, but also requisite spatial coordinates to organize the vascular connection between the fetus and its placenta. Indeed, via Brachyury, the primitive streak regulates placement of a unique but hitherto undocumented blood vessel, the 'vessel of confluence' (Daane et al., 2011; Downs et al., 1998; Inman and Downs, 2006), within the allantois (Rodriguez et al., 2017). As a result, the axially positioned vessel of confluence becomes a fixed branchpoint that, through patterning involving the fibroblast growth factor family, unites the umbilical, omphalomesenteric and fetal cardiovascular systems (Rodriguez et al., 2017). Mis-patterning the fetal–placental connection leads to severe birth defects (Rodriguez et al., 2017; Schreiner and Hoornbeek, 1973). The vessel of confluence and dense allantoic core are conserved across all Placentalia thus far examined, including humans (Rodriguez et al., 2017); conservation underscores the collective importance of these newly unearthed features in the fetal–placental connection.

Such fresh insights into the biology of the posterior embryonic–extra-embryonic region highlight the principle that, in Placentalia, the fetus does not make contact with its mother via its own devices. Rather, embryonic and extra-embryonic regions are unified through a common axial midline that organizes this relationship, and whose progenitor cells contribute to the fetal–placental interface.

Gene expression, potency and placental tissues

Not only does the allantois possess a source of progenitor cells used to build the placentae but so, too, do the amnion (Miki and Strom, 2006), chorion (Uy et al., 2002) and allantois-associated yolk sac (Rodriguez and Downs, 2017). All of these extra-embryonic tissues exhibit AlkP activity (Hahnel et al., 1990; MacGregor et al., 1995) which is found in most, if not all, stem cells (Benham et al., 1983; Bernstine et al., 1973). Despite its preponderance in the extra-embryonic region and throughout the epiblast, to the present day, AlkP activity is claimed to selectively identify PGC within the base of the allantois, associated visceral endoderm and the ventral component of the hindgut (reviewed in Mikedis and Downs, 2014). In addition to AlkP, other factors involved in the biology of stem cells have been found in extra-embryonic tissues. For example, *c-myc* is present in the derivatives of trophectoderm and in the base of the allantois (Downs et al., 1989) while OCT-3/4 has been identified in the ACD and adjacent yolk sac (Downs, 2008; Scholer et al., 1990).

Transcriptome analysis of posterior AlkP-positive cells led to the identification of BLIMP1 (PRDM1) and STELLA (DPPA3, PGC7) (Ohinata et al., 2005; Saitou et al., 2002), whose initial localization heralded these proteins as unique to the germ line antecedents (Ohinata et al., 2005; Saitou et al., 2002). Thus, on the basis of localization that was limited to a few stages and a few tissues in whole mount analyses,

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