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SPOTLIGHT

On the origin of the human germline

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ABSTRACT

In mice, primordial germ cells (PGCs), the precursors of eggs and sperm, originate from pregastrulation postimplantation embryos. By contrast, the origin of human PGCs (hPGCs) has been less clear and has been difficult to study because of the technical and ethical constraints that limit direct studies on human embryos. In recent years, however, *in vitro* simulation models using human pluripotent stem cells, together with surrogate non-rodent mammalian embryos, have provided insights and experimental approaches to address this issue. Here, we review these studies, which suggest that the posterior epiblast and/or the nascent amnion in pregastrulation human embryos is a likely source of hPGCs, and that a different gene regulatory network controls PGCs in humans compared with in the mouse. Such studies on the origins and mechanisms of hPGC specification prompt further consideration of the somatic cell fate decisions that occur during early human development.

KEY WORDS: Primordial germ cells, Gastrulation, Epiblast, Human development, Epigenetic resetting, Transcription factors, Signalling, Amnion

Introduction

Primordial germ cells (PGCs) – the founder cells of sperm and egg – are specified during early development, and subsequently develop into mature gametes, which, at fertilisation, generate a totipotent zygote. These cells, which together are referred to as the germline, thus provide an enduring link between all generations and are mediators of evolution. The genetic and epigenetic information transmitted to the zygote by the germline is crucial for human health and disease, so understanding how this lineage arises is of key importance. Furthermore, understanding whether environmentally induced epigenetic modifications can be transmitted transgenerationally in mammals remains an ongoing aim. Understanding how new genetic and epigenetic information is integrated into the germline over evolutionary time is also of significant interest.

In mice, PGCs originate from pregastrulation postimplantation embryos. Human PGCs (hPGCs) are also thought to originate in the posterior epiblast in pregastrulation embryos, although a recent study on non-human primate embryos has suggested that the nascent amnion, which itself develops from the postimplantation epiblast, could also be a site of PGC specification. This finding could reflect differences in postimplantation development between the two species. Indeed, in rodents, the epiblast forms an egg cylinder, whereas in human and many non-rodent species, embryos develop as bilaminar discs (Fig. 1). Whether these differences have an impact on early cell fate decisions warrants careful consideration. Grasping an understanding of this possible evolutionary divergence in the mechanism and origin of the germline and soma during early human development will be crucial for advances in regenerative medicine. Altogether, these studies are essential for understanding the organisation and development of early human embryos.

The transcription factor regulatory network controlling hPGC specification

The examination of authentic *in vivo* migrating and gonadal mouse PGCs (mPGCs) and hPGCs has revealed both conserved and unique features of the germ cell lineage between the two species (Tang et al., 2015). The conserved elements include germ cell specifiers [BLIMP1 (also known as PRDM1), TFAP2C (also known as AP2gamma)], germ cell factors (NANOS3, DND1, DDX4, DAZL) and pluripotency factors [OCT4 (also known as POU5F1), NANOG]. The conserved expression of these factors in mouse and human embryos does not, however, exclude species-specific mechanistic differences in their roles.

Besides these conserved elements, several unique features are evident in human and some non-rodent PGCs. Crucially, there is expression of SOX17 in the human germline, which is not seen in the mouse germline (Irie et al., 2015; Tang et al., 2015). Other differences include repression of SOX2 in the human germ cell lineage; by contrast, this key pluripotency factor is essential for the maintenance of mPGCs (Campolo et al., 2013). Furthermore, expression of KLF4, a naïve pluripotency factor, in hPGCs is noteworthy, as KLF4 is repressed in mPGCs by BLIMP1 (Durcova-Hills et al., 2008; Hackett et al., 2017). Interestingly, germ cells in pig and monkey embryos, which similarly develop as bilaminar discs, also share these characteristics of the human germline (Kobayashi et al., 2017; Sasaki et al., 2016). Even in basal Hystricognathi rodents, *Lagostomus maximus*, which surprisingly develop as flat-bilaminar discs, PGCs show expression of SOX17 and absence of SOX2 expression (Leopardo and Vitullo, 2017). These observations might indicate a correspondence between embryonic structure and the associated usage of crucial transcription factors. Future studies on diverse mammalian species might reveal the extent to which these characteristics are linked.

To investigate the mechanisms underlying hPGC specification in greater detail, we have recently developed *in vitro* models using human pluripotent stem cells (hPSCs), which are considered to be equivalent to early postimplantation epiblast cells, in an attempt to mimic posterior epiblast development during gastrulation (Irie et al., 2015; Kobayashi et al., 2017). These *in vitro* models identified SOX17 as a crucial specifier of hPGC fate, confirming our initial findings (Irie et al., 2015). Loss of SOX17 prevents hPGC specification definitively, whereas overexpression of SOX17 in cells that are PGC-competent induces hPGC characteristics without external signals (Irie et al., 2015). Although SOX17 gene dosage is

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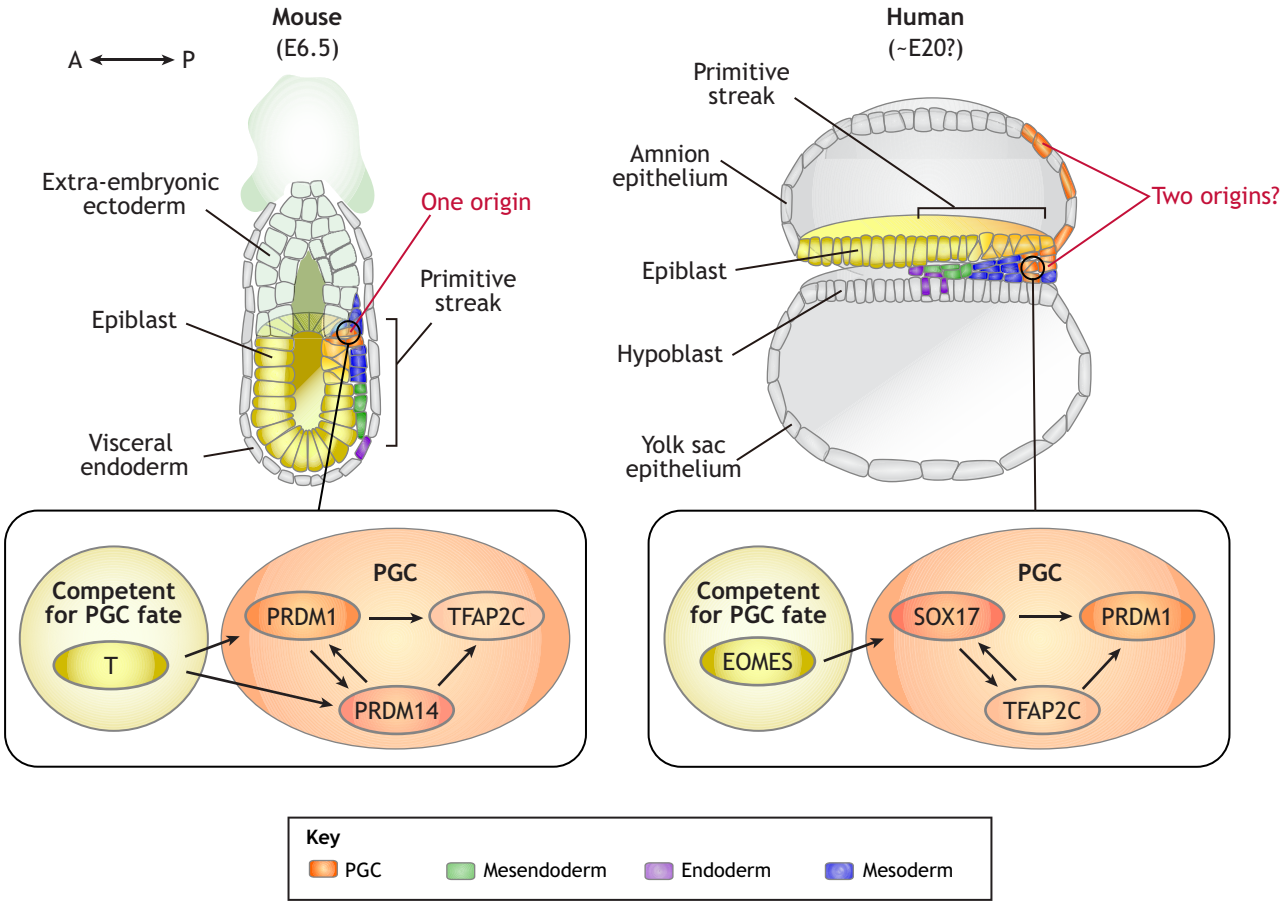

Fig. 1. The origin of mouse and human germline during gastrulation. Schematics of mouse and human gastrulating embryos, highlighting the potential sites of PGC specification, and crucial differences in the transcription factor networks controlling PGC specification. A↔P, anterior-posterior axis.

crucial, concomitant BLIMP1 expression is sufficient for robust induction of hPGC fate and initiation of epigenetic resetting (Kobayashi et al., 2017). SOX17 and BLIMP1 are the two factors with a central role in hPGC specification, but other factors, including TFAP2C, are also required (Kojima et al., 2017).

Notably, the expression patterns of SOX17 and SOX2 are mutually exclusive in hPGCs and hPSCs. Downregulation of SOX2 may be crucial for hPGC specification (Lin et al., 2014), but is not necessary for mPGC fate. In one potential model, it has been suggested that the downregulation of SOX2 may release OCT4 from playing a crucial role in pluripotency, and thus allow it to partner with SOX17 to initiate hPGC specification (Tang et al., 2016). Another recent study suggests that OCT4 partners with PAX5 and PRDM1 in hPGCs but not in hPSCs (Fang et al., 2018). On the whole, dynamic changes in protein complexes involving OCT4 are likely during hPGC specification. A little later, during gastrulation, SOX17 is likely to be the crucial inducer of definitive endoderm (DE) (Kobayashi et al., 2017). Further studies using *in vitro* models that simulate gastrulation could provide vital mechanistic information on how SOX17 is likely to induce two vital cell fates – first hPGCs and then DE shortly thereafter – in early human embryos.

The expression of another transcription factor, EOMES, occurs upstream of SOX17 in the PGC-competent human epiblast state (Kojima et al., 2017), and in the DE later (Teo et al., 2011). In contrast, brachyury (T, TBXT), a mesodermal factor and activator of mPGC fate (Aramaki et al., 2013), is not essential for hPGCs (Kojima et al., 2017). The conserved PGC specifiers BLIMP1 and TFAP2C are also among the first factors crucial for hPGC fate. However, the regulatory networks that these factors operate within, as deduced from the analysis of mutant cells, and their likely targets are not identical to those in mPGCs (Irie et al., 2015; Kojima et al., 2017; Sasaki et al., 2015; Tang et al., 2015). These features emphasise an evolutionary divergence of the transcription factor regulatory network in PGCs in mouse and human. Taken together with the transcriptomic analysis of *in vivo* PGCs and functional analysis using *in vitro* models, these findings reveal a distinctive transcription factor regulatory network for hPGC specification (Fig. 1). Continuing studies will show precisely how the conserved and unique transcription factors induce hPGC specification, and how the underlying mechanism differs from that controlling mouse PGC specification.

Signalling principles underlying hPGC specification

The analyses of mouse and pig embryos, and *in vitro* models inducing the formation of hPGCs from hPSCs, have shown that the action of WNT and BMP is crucial and conserved for PGC fate across the mammalian species (Kobayashi et al., 2017; Ohinata et al., 2009). WNT signals are known to be necessary for the identity of the posterior epiblast, and subsequent gastrulation and mesoderm formation. Thus, activators of WNT signals are abundant in the posterior epiblast. BMP signals are also crucial inducers of PGC fate. Although primitive endoderm derivatives (the visceral endoderm in rodents, the hypoblast in non-rodents) are a source of BMP2, non-rodents notably lack extra-embryonic ectoderm,

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which aligns physically with the proximal epiblast and is a significant source of BMP4 in the mouse. In non-rodent mammals, such as rabbit and pig, it has been shown that the posterior epiblast starts to express WNT at the pre-primitive streak stage. Afterwards, the posterior epiblast and incipient mesoderm begin to express BMP4 at an early primitive streak stage during the onset of gastrulation (Yoshida et al., 2016).

In line with these well-established signalling principles, we found that porcine PGCs are specified at the posterior epiblast at the early primitive streak stage, suggesting the posterior epiblast has an appropriate environment for PGC induction (Kobayashi et al., 2017). However, a recent study of cynomolgus monkey embryos has indicated that nascent PGCs can emerge from the early amnion, which is an extra-embryonic membrane structure that subsequently surrounds the fetus (Sasaki et al., 2016). In primates, including humans, the amnion is formed from the pluripotent epiblast soon after implantation, albeit at a different time; this is not the case in pig and other mammalian embryos, in which the amnion develops after the initiation of gastrulation (Hassoun et al., 2010). In monkey embryos, WNT is expressed in the cytotrophoblast layer relatively close to the amnion, whereas the amnion itself is a source of BMP, indicating that there is an appropriate environment in this tissue for PGC specification (Sasaki et al., 2016). Regardless, the question of whether PGCs originate exclusively from the amnion in primates still remains unanswered.

On the origin of human and non-human primate PGCs *in vivo*

The sequential expression of WNT and BMP signals is expected to occur in the posterior epiblast in monkey embryos, as in other mammalian embryos, from the onset of gastrulation onwards. Other hallmarks of competency for PGC fate include expression of genes such as *T* and *EOMES*, which are observed in the nascent amnion (Kojima et al., 2017; Sasaki et al., 2016). These are also the functional transcription factors in the primitive streak at gastrulation across the mammalian species. Importantly, for the *in vitro* models using monkey and human PSCs, the induction of a posterior epiblast state in pregastrulation embryos is a prerequisite for PGC specification and marks a transient state that is competent for PGC fate (Kobayashi et al., 2017; Sasaki et al., 2015). Accordingly, continuing WNT and activin signalling in our *in vitro* model causes the loss of the transient PGC-competent state, and the cells progress towards mesendoderm – a precursor of mesoderm and endoderm (Kobayashi et al., 2017). Consequently, the posterior epiblast in pregastrulation embryos is likely to be an appropriate source of PGCs in primates.

How is the signalling for inducing hPGCs in the epiblast compatible with the amnion also acting as a potential site for the origin of PGCs in non-human primates? As mentioned above, the amnion in primates develops from the postimplantation epiblast, albeit at different times; early in humans (soon after implantation on ∼day 7) and relatively late in Rhesus monkey (∼day 9-10 after implantation), which belongs to the same genus (*Macaca*) as cynomolgus monkey (Luckett, 1975). The derivation of amniotic cells from the postimplantation epiblast could result in this lineage retaining the essential characteristics of early postimplantation epiblast cells. Indeed, it has been shown that nascent amniotic cells maintain expression of the pluripotency factors OCT4, NANOG and SOX2, with subsequent downregulation of SOX2, and that the repression of OCT4 and NANOG follows after the specification of PGCs (Sasaki et al., 2016). This sequence of gene expression is reminiscent of that observed in the developing posterior epiblast of pregastrulation embryos. As the signalling principle orchestrating PGC fate appears to be similar in the epiblast and in the amnion, it is possible that hPGC specification occurs at two sites in primates, the amnion and the posterior epiblast, although this requires validation. Comparing the molecular and functional properties of the amnion with those of the epiblast will, therefore, be crucial to confirm the fundamental basis of the PGC-competent state in the amnion. For example, single-cell transcriptomic analyses of nascent amnion and its subsequent development might reveal whether there are similarities to the posterior epiblast at the time of pregastrulation development. Lineage tracing of PGCs from the amnion will also be essential to determine whether there is a single or dual origin of primate PGCs. In this regard, a recent study on the self-organisation of amnion-like cells from hPSCs suggests a resemblance to the observation in the monkey embryo, which might provide an opportunity to address the origin of PGCs from these cells *in vitro* (Shao et al., 2017a,b).

Conclusions

Understanding the specification and origin of PGCs, in addition to that of the three somatic lineages that occur in gastrulating human embryos, is pivotal for advances in regenerative medicine. In recent years, the focus of substantial research has been on the derivation of diverse cell types from hPSCs. However, a comparison of the mechanisms that regulate human and mouse somatic and germline cell fates during gastrulation has provided key insights into early human development. There appears to be differences in the transcription factors regulating pluripotency in mouse and human, and in postimplantation bilaminar disc epiblast development (Rossant and Tam, 2017). The development of a bilaminar disc at gastrulation is not confined to the non-rodent Eutherian mammals, as it also occurs in marsupials, and indeed other vertebrates such as chicken (Sheng, 2015). This broad evolutionary conservation is noteworthy, and its overall impact on cell fate decisions in non-rodents deserves consideration.

It should also be noted that there is much greater developmental diversity in the extra-embryonic tissues of mammals compared with the epiblast. In this context, a putative origin of PGCs from the extra-embryonic amnion in primates needs consideration. A dual origin of hPGCs from the posterior epiblast and amnion is a possibility, which would be reminiscent of the source of haematopoietic stem cells, i.e. from the embryonic (lateral plate) and extra-embryonic (yolk sac) mesoderm derivatives (Costa et al., 2012). Further refinement of *in vitro* 2D and 3D models of early human embryos from hPSCs is possible, although studies on the development of human blastocysts in culture will require technical advances and lifting of the regulatory restrictions (Deglincerti et al., 2016; Shahbazi et al., 2016). Future investigations should also address the diversity of early mammalian development, and its impact on cell fate decisions, which together will provide the context for determining the crucial aspects of early human development.

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Competing interests

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