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ADHESION MOLECULES FOR MOUSE PRIMORDIAL GERM CELLS

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1. ABSTRACT

In the present article we will focus on the adhesion molecules expressed by mouse primordial germ cells (PGCs) and will discuss the role that they play, or are believed to play, in two crucial processes of PGC development, namely cell lineage specification and migration into the gonadal ridges. Recent findings indicate that the adhesion-dependent allocation of the PGC precursors to a niche within the epiblast and the forming extraembryonic mesoderm during the pre-gastrulation period is crucial for their commitment. Subsequently, PGC migration and homing within the gonadal ridges require integrated signals involving contact of PGCs with extracellular matrix molecules and cellular substrates or repulsion from them, adhesion among PGCs themselves and attraction by the developing gonads. A number of adhesion, or putative adhesion molecules, have been identified in mammalian PGCs, mainly in the mouse. These molecules belong to three adhesion molecule families such as cadherins (E-P- and N-cadherins), integrins and the IgG superfamily (PECAM-1). Moreover oligosaccarides (LewisX) and growth factor receptors (c-Kit) can also play adhesive roles in some stages of PGC development. An understanding of how genes encoding adhesive molecules are regulated in PGCs and the molecular pathways associated with the functions of adhesion receptors is crucial in furthering our knowledge of PGC biology. Adhesion molecules might once again turn out to be crucial in controlling not only the germ cell lineage and PGC migration but also the PGC differentiation fate itself.

2. INTRODUCTION

During the last years, the development of mammalian primordial germ cells (PGCs), the embryonic precursors of the eggs and sperm, has been an area of growing interest for scientists aspiring not only to unravel the early stages of gametogenesis, but also to understand the unique ability of PGCs to maintain the differentiation totipotency necessary to give rise to gametes. In the present review we will focus on the adhesion molecules expressed by mouse PGCs and will discuss the role that they play, or are believed to play, in two crucial processes of PGC development, namely cell lineage specification and migration into the gonadal ridges. The PGCs of all vertebrates studied arise outside the gonads and reach them later in development by active migration. The exact route taken may differ among species, but the general principle of the establishment of germ line cells in an extraembryonic site, followed by their active movement to the developing gonad, is now well established. Some important new findings about how these events occur in mammals have been recently obtained. As in others developmental processes, cell to cell and cell to extracellular matrix (ECM) adhesion are involved and play a major regulative role. A number of adhesion, or putative adhesion molecules, have been identified in mammalian PGCs. However, their precise role/s and the adhesion-associated signal transduction events remain mostly unknown. Difficulty in accessing mammalian PGCs within the embryo, in defining and employing suitable *in vitro* experimental models and applying molecular manipulation techniques to PGCs, have so far slowed down and limited the progress in this field. The main results that have been obtained so far on this subject, mostly in the mouse, together with working hypotheses and future perspectives, are object of the present review.

3. ADHESION MOLECULES IN PGC COMMITMENT: ARM IN ARM TO FIND A LINEAGE

In the mouse embryo, the PGCs arise from precursors located in the rim of the proximal epiblast. At 6-7 dpc (days post coitus) the PGC precursors move from the epiblast to the posterior edge of the primitive streak where the germ cell lineage is established. PGCs then migrate into the endoderm of the developing hindgut and eventually into the gonadal ridges (10.5-12.5 dpc). At 12-13 dpc, PGCs differentiate into oocytes in the ovary and into prospermatogonia in the testis. Similar developmental stages are followed by PGCs in all mammalian species studied, including humans (for reviews, see 1-5).

Recent findings indicate that the formation of mouse PGCs from proximal epiblast requires multiple

steps, involving growth factor signalling, cell-cell interaction and cell movement. The specification of the germ cell lineage has been object of recent reviews (6-8) and will not be discussed in detail here. We will focus on the adhesive interactions that appear to be involved in this process. The first signals by bone morphogenetic protein (BMP) growth factors, necessary to commit epiblast cells towards the extramesodermal fate in which PGC precursors are established, require that cells of the proximal epiblast remain closely packed while moving to the posterior end of the primitive streak. This was established by the finding that induction of PGCs was much more efficient in cultured epiblast fragments containing at least 40 cells or in closely packed epiblast explants than in dissociated epiblast cells (9, 10). In the forming extraembryonic mesoderm, adhesion among PGC precursors might be also crucial for PGC specification. Proteins possibly involved in such cell-to-cell adhesion might be encoded by members of a family of interferon-inducible genes called *fragilis* (*fragilis*/*mil1*, *fragilis2*/*mil2* and *fragilis3*/*mil3*; 11, 12). Saitou *et al.* (11) showed that cells expressing high level of *fragilis* gather together in the posterior primitive streak and allantois of 7-7.5 dpc late streak embryos. In this region, some of the *fragilis*-expressing cells begin to express *stella* (also called *PGC7* and *Dpppa3*;11). Expression of *stella*, which encodes a putative chromosomal organization or RNA processing factor, marks the emergence of a committed PGC populations (11,13). One prototype member of these Fragilis proteins in humans is the 9-27 protein in leukocytes and endothelial cells, identical to the Leu-13 antigen. This protein is not itself an adhesion molecule, but is part of a multimeric protein complex that upon activation is able to transduce antiproliferative and homotypic adhesion signals (14).

Another adhesion molecule that appears to play a role in the allocation of PGC precursors is the classical epithelial adhesion molecule E-cadherin. Okamura and coll. (15), have recently shown that around 6.75-7.25 dpc E-cadherin is expressed in cells of the proximal extra-embryonic mesoderm where the PGC precursors are located and that, at least *in vitro*, adhesive interactions mediated by E-cadherin are crucial for these precursors to become PGCs. According to the observations by Anderson *et al.* (16), using specific PGC green fluorescent (GFP) marker, at 7.5 dpc mouse PGCs are already highly motile and migrate directly from the posterior end of the primitive streak into the adjacent embryonic endoderm. These authors also claim that the clusters of GFP+/Alkaline Phosphatase+ cells located in the extraembryonic mesoderm of the proximal allantois at 7.5-8.5 dpc, believed to be presumptive PGCs (17), do not actually contribute to the germ cell population. On this basis, it appears that PGCs are established in a narrow temporal window between 6.75 and 7.25 dpc in the posterior end of the primitive streak and once a cluster of nascent PGCs is formed, expression of adhesion molecules mediating PGC-PGC adhesion is rapidly down-regulated to allow them to actively migrate into the developing hindgut. Indeed, in PGCs located in the hindgut around 8.5-9.5 dpc the expression of *fragilis* and E-cadherin decreases or is absent, respectively (11, 18).

Several mechanisms by which cell-to-cell adhesion may control PGC formation are possible. For example, interaction mediated by E-cadherin might transmit intracellular signals by sequestering β-catenin from lymphoid enhancer factor/T cell factor (LEF/TCF) and regulating the expression of specific genes such as those needed to maintain totipotency (for a review, see 19). Interestingly, Wnt/β-catenin signalling has recently emerged as a key factor in controlling stem cell self-renewal and expansion (20). The relevance of Wnt/β-catenin in PGC development should certainly be investigated in future studies. Another possibility is that adhesion molecules might facilitate the clustering of receptors for growth factors critical for their efficient signalling in PGC determination or directly activate signalling molecules crucial for such process (*e.g.* BMPs or perhaps interferons; for a review, see 8). Alternatively, adhesion molecules might simply anchor the PGC precursors into a niche within the proximal epiblast and the extraembryonic mesoderm for a time sufficient to allow them to respond to local inductive signals or to protect them from signals leading the surrounding cells into somatic differentiation lineages.

4. ADHESION MOLECULES IN PGC MIGRATION: MOVING TOWARD THE FINAL DESTINATION

As reported above, once formed, mouse PGCs enter by active migration into the embryonic endoderm (between 8 and 9 dpc) that will give rise to the hindgut (16). For about one day, PGCs remain as stationary cells within the hindgut epithelium, thereafter from 9.5-11.5 dpc, they actively migrate toward the gonadal ridges, displaying motile features (21). What tells the PGCs when to start moving, where to move and when and where to stop? What are the regulators of cell contacts and cytoskeletal dynamics that underlie the cell motility changes in PGCs? Stage-dependent expression of adhesion molecules and surface glycoproteins, which will be described in the next sections, appear to accompany these processes. The significance of such changes and the molecular signal(s) responsible for the start of PGC migration are, however, unknown. Little information is also available about the molecular mechanisms governing the motility machinery of PGCs and the signals that guide them along specific migratory pathways. Similarly to *Drosophila* and *Zebrafish,* in which mutations have allowed a best characterization of the molecular mechanisms of PGC migration (for a review, see 22), we may hypothesise that a balance between attractant and repellent molecules secreted by the surrounding cells result in mobilization of PGCs and direct them toward the gonadal ridges. Central in cell migration is the fascinating ability of cells to detect shallow gradients of extracellular molecules and to link that sensing to changes in cell morphology and motility. These capabilities are essential for cell polarization and chemotaxis that guide cells toward attractants or away from repellents. For several decades, it has been postulated that gonadal ridges produce chemoattractants for PGCs and some evidence for this has also been obtained in the mouse (23). The search for chemoattractants for mouse PGCs is, however, still ongoing. Possible candidates are transforming growth factor β (TGFβ, 23-25), kit ligand (KL), also known as stem cell factor (SCF) (26) and

Table 1. Adhesion molecules expressed by mouse primordial germ cells as a function of their developmental stages.

Adhesion Molecules		Embryo Age (dpc)			
		8.5	10.5-11.5	12.5	13.5
Integrins	α3	ND	+	-	-
	α5	ND	+	+	+
	α6	ND	±	++	++
	αV	ND	++	++	++
	β1	ND	+	+	+
	β3	ND	++	++	++
Cadherins	E-caderin	-	+	+	±
	P-caderin	-	+	+	ND
	N-caderin	-	-	±	+
IgG family	PECAM-1	+	+	+	+
Sugars	LewisX	-	+	+	±
Others	KL/c-Kit	+	+	+	±
	EpiCAM	ND	+	+	+

ND = not determined

stromal-cell derived factor-1 (SDF-1, 27-28), but robust proof in favour of the chemoattractive role for any of these growth factors is lacking. In any case, for proper movement migratory cells need suitable substrates. Besides cell motility, extracellular matrix (ECM) and cellular substrates may be necessary for directional movement and to provide migratory cells with molecules needed for their survival and growth. For this reason, the search for adhesion molecules mediating interactions of PGCs with ECM molecules and the surrounding cells has been, and still is, an active field of research **table 1**.

4.1. Walking on sticky and slippery carpets

Adhesion to ECM molecules are among the most important factors determining a cell's migratory capability. Early morphological studies in the mouse embryo indicated that PGCs migrate along a discontinuous basal lamina underlying the coelomic epithelium (21), in which type IV collagen (CIV), laminin (LM) and fibronectin (FN) were identifiable (29, 30). FN and LM were also found around the mesenchymal cells between which PGCs migrate (30, 31) and the somatic cells of the undifferentiated gonads (29, 32). In this regard, LM, but not FN and CIV, was found in high concentration in the developing 11.5 dpc gonadal ridges, arranged as a discrete layer surrounding PGC clusters (33). More recently, Soto-Suazo *et al.* (34, 35) identified hyaluronan, collagen I, III and V and tenascin surrounding migratory mouse PGCs. Several years ago, the possibility to first isolate and culture mouse PGCs from 8.5 to 13.5 dpc (36, 37), allowed us to begin to investigate some aspects of their capability to adhere to and move on ECM molecules (31, 38) figure 1.

In functional studies, using an explant culture system, Ffrench-Constant *et al.* (39) found that 9.5 dpc PGCs emigrate from tissue fragments containing hindgut onto a supporting fibroblast STO cell monolayer and exogenous FN stimulates such migration. All these observations indicate that PGCs may encounter a variety of ECM molecules potentially able to permit or inhibit their displacement during migration. Moreover, PGCs change their adhesion properties to some ECM molecules as a function of the developmental stage. In particular, it appears that migratory PGCs do not adhere to glycosaminoglicans (GAGs) and collagen type I (CI), while they attach with relatively low strength to FN as expected for migratory cells whose movement is facilitated by a hydrated environment (GAGs) and needs dynamic adhesion and displacement from the substrates.

It is possible instead that the higher ability of PGCs to attach to LM and CIV prevent them passing through the forming basal lamina of the coelomic epithelium as demonstrated in Xenopus (40). The accumulation of LM around PGCs during the early period of gonad colonization and the concomitant transitory increase in PGC adhesion to this protein, suggest that LM may eventually contribute to PGC settlement in the gonadal ridges. It is to be pointed out that these adhesiveness studies reported a limited ability by PGCs to display features of motility on all ECM substrates tested.

In an effort to identify integrin receptors responsible for PGC adhesion to ECM molecules, the expression of integrin subunits by migratory and post-migratory PGCs has been analysed (38, 41, 42). PGCs were found to express high levels of integrin subunits αV and β3; expression of integrin subunits β1, α6 and low levels of α5 and α3 (at 10.5 dpc only) were also detected table 1. The laminin-binding heparan sulphate proteoglycan-α-dystroglycan was found expressed by migratory and post-migratory PGCs (43). Interestingly, while some integrins such as αV, α5, β1 and β3 are expressed at constant levels by migratory (10.5-11.5 dpc) and post-migratory (12.5-13.5 dpc) PGCs, the expression of others (α3, α6) appears to be modulated. Such distinct integrin expression is in accord with the stage-dependent adhesiveness of PGCs to different ECM molecules reported above and is likely to be related to changes in affinity for the various ECM molecules encountered during migration. While the continuous presence of the dimers α5β1 and αVβ3 on the PGC membrane may assure a constant capability to interact with FN (the ligand for both these receptors) during the entire migratory period, the higher expression of α6, mainly in the period of PGC settlement into the gonadal ridges, and

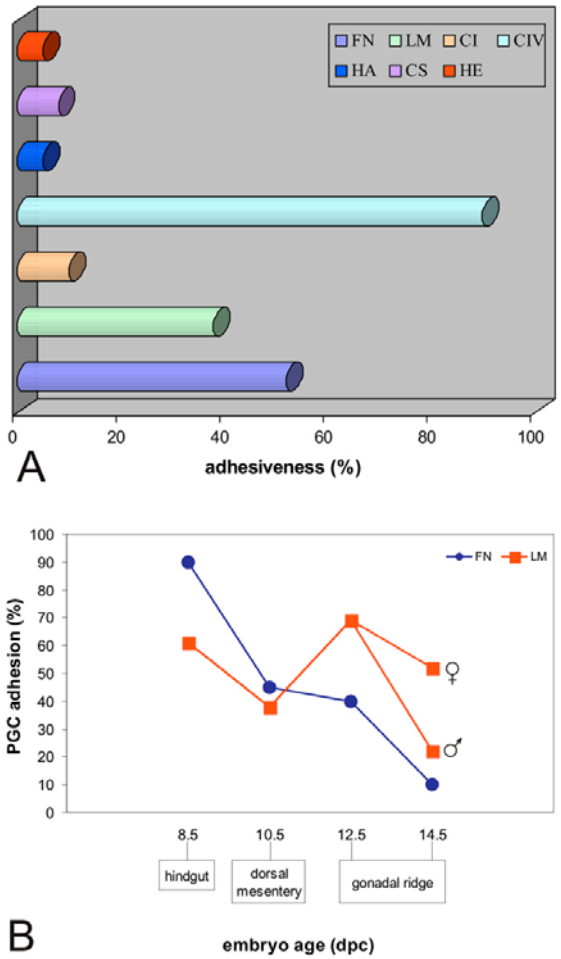

Figure 1. Adhesion of mouse PGCs onto extracellular matrix molecules *in vitro*. A. Adhesion of 10.5-11.5 dpc migratory PGCs onto Fibronectin (FN), Laminin (LM), Collagen type I (CI), Collagen type IV (CIV), Hyaluronic acid (HA), Chondroitin sulphates (CS) and Heparin (HE). B. Changes in the adhesiveness of PGCs to FN and LM as a function of their developmental stage and localization in the embryo. The results reported were obtained by combining the studies of references 33 and 38.

the presence of its partner β1, might be related to the specificity of the dimers α6β1 for LM. Among the expressed integrin subunits, β1 is necessary for PGC adhesion to FN as expected (38), but surprisingly enough, it is the only one whose ablation seems to cause impaired gonadal ridge colonization. At 13.5 dpc only 40% of the $\beta1^{-}/\beta1^{-}$ PGCs are located within the gonadal ridges while the remainder are scattered in ectopic sites (42). Do these ablation experiments mean that most of the integrin subunits found on the PGC membrane are actually not required for their migration? This is unlikely, since it is possible, as it occurs in other cell types, that in the absence of one integrin subunit, PGCs up-regulate the expression of other subunits. Multiple gene targeting of integrins expressed by PGCs will be needed to draw firmer conclusions. Important information which is also completely lacking about PGC integrins regards their dynamic redistribution upon adhesion to a substrate and the intracellular signalling linked to their activation crucial in other cell types (for a review, see 44). Whether integrin expression in PGCs is regulated by some of the growth factors known to act on PGCs during this period (for a review, see 3) would be also interesting to investigate.

Some integrins normally involved in lymphocyte homing and leukocyte extravasation (for a review, see 45), are able to mediate cell-cell adhesion by binding to cadherins (46) or to the IgG superfamily of adhesion molecules (47). No evidence for the presence of such adhesion systems in PGCs has been reported, although members of the cadherin (P-cadherin, 48 see below) and the IgG (NCAM, 49) family of adhesion molecules have been found in the mouse gonadal ridges.

Whatever the precise role and mechanism of PGC interaction with ECM molecules, a theme arising from these studies is that adhesion to ECM components is certainly important for PGC migration as expected, but is insufficient alone, at least in the *in vitro* situation, to fully activate and/or maintain the motility machinery of PGCs. It is likely, as we will see in more detail in the next section, that signalling from integrin receptors must cooperate with that of other cell-to-cell adhesion molecules and of more traditional signalling receptors, such as growth factor receptors, to assure a proper PGC migration and survival.

4.2. Walking among supporting cells

Migratory mouse PGCs have been found to establish transitory close contact with the somatic cells (mostly mesenchymal cells and coelomic mesothelial cells) they encounter along their migratory pathway (21). It is not clear, however, whether this has any relevant role for their migration and homing. Several cell feeder layers have been used which support PGC adhesion to different degrees *in vitro* (50-52). In general, migratory PGCs attached moderately to a cell monolayer and show features of motile cells with hallmarks of invasive cells figure 2A (51, 53). This is in accord with the highly dynamic nature of adhesive interaction by migratory cells and suggests that multiple signalling from adhesive molecules of ECM, the cellular substrate and soluble factors are necessary to fully stimulate PGC motility. The interpretation of these *in vitro* observations, however, is complicated by the fact that PGCs are dependent for survival on growth factors produced by the surrounding cells (see below and the review by 3). It is therefore difficult to discriminate if PGC motility observed in such cultures is an indirect effect resulting from the survival factors produced by the cell feeder layers. The development of a migration assay in which PGCs could be studied without somatic cell monolayers might help to answer some of these questions. Nevertheless, these *in vitro* observations confirmed that PGCs may migrate to the gonads by active locomotion, a feature definitively established by the recent studies in which the migration of live GFP-stained PGCs in the mouse embryo was observed (16, 54).

PGC adhesion to somatic cells, besides necessary for fully activating their motility machinery, might have a

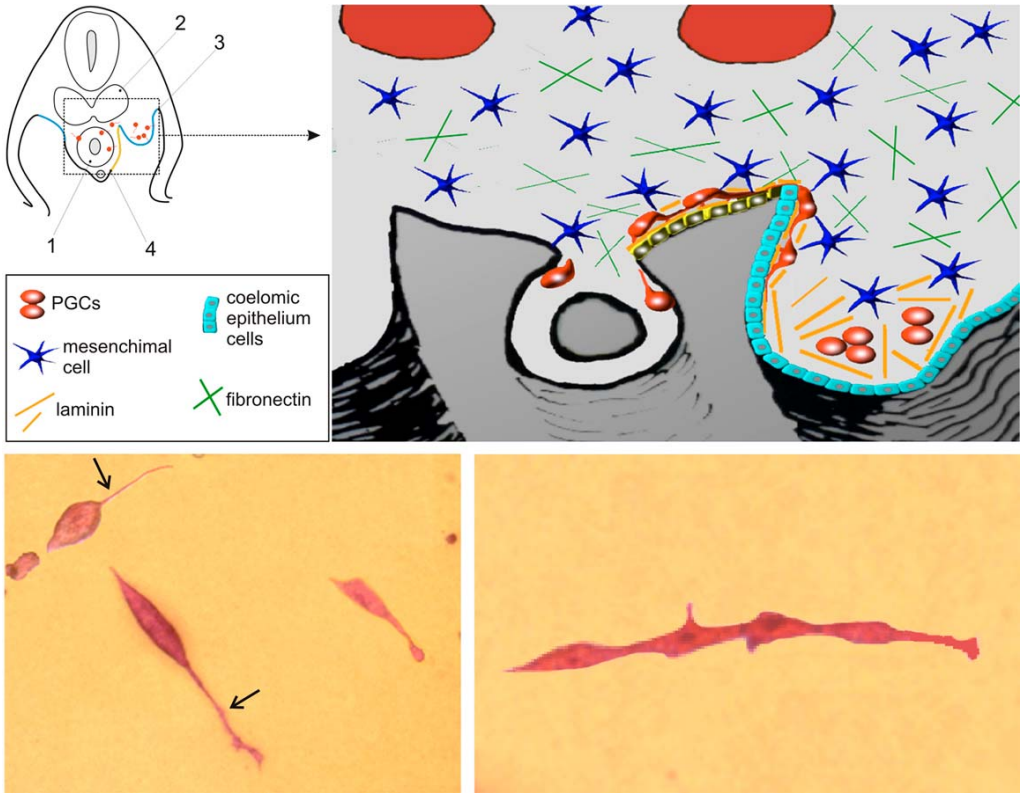

Figure 2. Migration of mouse PGCs toward the gonadal ridges. A: schematic drawing showing the position of PGCs between 9 and 10 dpc. The drawing on the right recapitulates the main adhesive interactions that migrating PGCs may establish with ECM molecules (namely fibronectin and laminin) and the surrounding somatic cells (mesenchymal and coelomic epithelium cells). 1 = hindgut, 2 = dorsal aortae, 3 = gonadal ridges, 4 = coelomic wall. B: single PGCs and a cluster of four PGCs from 11.5 dpc embryos observed after about 24 hr of culture *in vitro*. Cells were stained for alkaline phosphatase activity. Arrows indicate leading filopodia; filming and interference reflection studies (69), show that PGCs in culture actively move at a speed of around 50 μm/hr.

role in directing them to the gonadal ridges and eventually anchoring them within the gonads. An adhesive molecule could be present in increasing amounts in the somatic cells along the migratory pathway of PGCs and in the gonadal ridges. A PGC that is constantly making and breaking adhesion with such a molecule would move from a region of low concentration to an area where the adhesive molecule is more concentrated, a process called haptotaxis. The membrane bound form of the KL growth factor might exert such a role. Keshet *et al.* (26) describe a gradient of KL expressed along the path of migratory PGCs and suggest that this may provide a mechanism for the control of their homing. Godin *et al.* (25) showed that soluble KL did not exert a chemotropic effect on mouse PGCs *in vitro*, but it was able to stimulate their motility. The analysis of migration in W^e/W^e PGCs carrying a mutation at the *W* locus resulting in a nonfunctional form of the KL receptor c-kit, showed that by 10.5 dpc most of the PGCs formed clumps and remained in the gut wall unable to migrate (55). Moreover, we found that KL/c-kit interaction contributes to the adhesion of PGCs to somatic cells in culture (56). Since KL/c-kit also exerts an anti-apoptotic action on PGCs (57), it is possible to postulate an elegant mechanism for the control of the number and position of PGCs based on the elimination of PGCs that stray from the migratory route. Among the many proteins implicated in the regulation of cell survival Akt/PKB is a likely target for the adhesion-dependent c-kit activation in PGCs (58, 59). The molecular pathway downstream of Akt activation (phosphorylation) in PGCs is largely unknown, although down-regulation of the pro-apoptotic Bax protein might be one of the targets (60, 61).

No other adhesive molecules have been identified on the surface of embryonic somatic cells which are candidates to influence PGC migration. On the other hand, it is also doubtful that PGC settlement in the gonad is solely due to a selective recognition of and adhesion to the somatic cells of the gonadal ridges. In fact, PGCs are able to attach *in vitro* to cell monolayers of various cell types (62). Moreover, in an *in vitro* assay originally devised for lymphocyte/endothelial cell adhesion (63), PGCs were found to adhere to sections of tissues obtained from various embryonic organs (12.5 dpc gonad, heart and brain) although not from liver and kidney (our unpublished observations).

Although there is scant evidence that embryonic somatic cells express distinct adhesion molecules for PGCs, a number of members of the major families of cell-cell adhesion molecules are expressed by PGCs. Several

glycoconjugates are present on the PGC surface as revealed by their ability to bind a variety of lectins (64, 65) and the presence of an abundant glycocalyx both in rodent and human PGCs (for references, see 66). In particular, PGCs express 3-fucosyllactosamine (or Lewis-X, Le^x), also know as the oligosaccharide antigen SSEA-1, TG-1, EMA-1 or 4C-9 (51). This oligosaccharide moiety is present on the surface of mouse and human PGCs only during migration. It also expressed on early blastomeres of mouse embryo, where it is thought to play a role in the homotypic cell-cell adhesion responsible for embryo compactation (67). In leukocytes, Le^X and the closely related sialyl Le^x are ligands for selectins, a protein family of adhesion receptors mediating oligoasaccharide-dependent leukocyte adhesion to endothelium and extravasation (for a review, see 45). Interestingly, Le^X is also expressed in embryonic pluripotent cells and some adult stem cells (i.e. neuronal stem cells), in which a role in promoting growth factor oligomerization-dependent self renewal and Wnt signalling has been suggested (68). There is not unequivocal evidence that Le^X is used as an adhesive molecule by PGCs, although early studies carried out by us and others (62, 69) showed that anti-SSEA1 or EMA-1 antibodies partly blocked the initial adhesion of PGCs to certain cell monolayers. Moreover, there is no information on the presence of selectins in the somatic cells of the gonadal ridges. On the other hand, PGCs themselves could express selectins or selectin-like molecules, since they show the ability to attach to dishes coated with specific bovine serum albumin (BSA)-linked sugars *in vitro* (e.g. phosphate-galactose and α-mannose; our unpublished observations). In this regard it is worth mentioning that prenatal exposure to high galactose impaired PGC migration in rat embryos, leading to the development of gonads with highly deficient pools of germ cells (66).

Another adhesion molecule expressed by migratory mouse PGCs is PECAM-1 table 1 (70, our unpublished observations). PECAM-1 is an adhesion molecule of the IgG adhesion molecule superfamily and participates in both homophilic and heterophilic adhesion during leukocyte-endothelial transmigration (71) and activation of integrins on leukocytes and T cells (72, 73). It can bind PECAM-1 itself (74), proteoglycans (75), the αvβ3 integrin (76, 77) and CD38 (78). Which is the function on the PGC surface of an adhesion molecule typical of the hematopoietic system? Interestingly, in some species like birds and at least in one mammalian species, with large-sized embryos, the cow (79), PGCs reach the gonads through the blood and subsequently emigrate from the blood stream into the gonadal ridges. Therefore it is possible that adhesion systems normally used in leukocyte/endothelial cell interaction are conserved in PGCs. Whether they use PECAM-1 for adhesive interactions or for other functions is an issue that probably deserves further investigation.

4.3. Walking arm in arm towards an attractant

To further complicate this picture of the adhesion molecules, we and others identified members of the cadherin family present on the PGC surface table 1. Mouse PGCs express P-and E-cadherin during, and for a couple of days after migration, and N-cadherin at post–migratory stages. N-cadherin and P-cadherin function might be related to interactions of germ cells with somatic cells. In fact the former is expressed only by post-migratory PGCs in focal contact with somatic cells (18) and the latter is present in the somatic cells of sex-indifferent mouse gonads and the pre-Sertoli cells of the testis cords (48). E-cadherin is not expressed by PGCs in the hindgut, but is up-regulated as they leave it (18, 80). Since E-cadherin is not expressed in cells contacting migratory PGCs or in the gonadal somatic cells, it is unlikely that it plays a role in the interaction between PGCs and their supporting cells. It is more likely that E-cadherin mediates PGC-PGC interaction or recognition. The notion that PGC-PGC adhesive interactions might be actually important during migration was first indicated by a surprising property of migratory PGCs evidenced during *in vitro* culture experiments. PGCs isolated from the dorsal mesentery at 10.5 dpc and cultured on fibroblast monolayers are motile and contact each other through long thin processes, forming clusters of several cells figure 2A. Over time, clustered PGCs become closely apposed and round up, losing any motile phenotype (81). In line with these observations, PGCs isolated from 12.5-13.5 dpc gonads do not show any features of motile cells *in vitro* (51). These studies together with similar *in vivo* observations (81), raised the possibility that germ cell-germ cell contacts are necessary to link together PGCs during migration and have a role in switching off the migratory phenotype upon arrival within the gonadal ridges. According to these observations, a possible hypothesis is that accumulation of PGCs within the gonads involves a first wave of colonization by pioneer PGCs guided to the target by an attractant(s) and subsequent aggregation of interconnected PGCs. More recent observations of living GFP-stained PGCs in the mouse embryo showed that some PGCs are capable of independently migrating towards the gonadal ridges (54). Therefore, it is not possible to assess with certainty the relative importance of coalescence and individual migration in such a process. It is likely that both mechanisms are involved, perhaps in different periods of migration. But does E-cadherin play any role in PGC-PGC aggregation? Cadherins are believed to function in sorting cells during morphogenesis and in the establishment of tissue architecture and cell identity (for a review, see 82). As we have seen in a previous section, the precursors of PGCs express E-cadherin but around 9-9.5 dpc, PGCs in the hindgut are negative for this molecule. Hence it is possible that PGCs might need to have E-cadherin turned off in order to maintain a distinct identity within the hindgut epithelium. *De novo* expression of E-cadherin as they leave the gut might appear quite singular. In fact, E-cadherin is generally associated with the stationary phenotype of epithelial cells. Recent observations indicate, however, that it is also expressed by non-epithelial cells and that migratory cells express functional cadherins which are in some cases important for their oriented migration (83-85). We proved that E-cadherin is actually able to mediate homotypic PGC-PGC adhesion and that adhesion of migratory PGCs to E-cadherin expressing cell monolayers stimulates their motility (80). On this basis, we hypothesized that E-cadherin-dependent adhesion is an

important factor linking together migratory PGCs in the clusters observed by Gomperts and colleagues (81). Moreover, after PGCs reach the gonadal ridges, changes in E-cadherin functionality might have a role in their settlement at the stationary stage (80). In this regard, it is to be mentioned that mouse migratory PGCs also express Ep-CAM, an epithelial adhesion molecule not structurally related to any of the major families of the adhesion molecules (86). Ep-CAM negatively modulates E-cadherin-mediated adhesion by disrupting the link between α-catenin and F-actin and rendering relatively weak the link among cells expressing both molecules at the same time (87). This is in line with the dynamic adhesion needed among migratory cells and could represent a way to modulate the E-cadherin-mediated PGC adhesion during migration.

5. CONCLUSIONS

PGC migration from their site of origin to the gonadal ridges is regulated by a variety of interactions and molecules figure 2B. Among these a major role is certainly played by adhesion molecules expressed by PGCs. Currently, identification of molecules that are necessary for PGC lineage determination and migration is an area of active research. Recent findings indicate that the adhesion-dependent allocation of the PGC precursors to a niche within the epiblast and the forming extraembryonic mesoderm during the pre-gastrulation period is crucial for their commitment. Subsequently, PGC migration and homing within the gonadal ridges require integrated signals involving contact of PGCs with ECM molecules and cellular substrates or repulsion from them, adhesion among PGCs themselves and attraction by the developing gonads. The relative importance of these mechanisms remains to be clearly established. They are not mutually exclusive and their relevance might change over time according to different conditions of PGC migration. As highlighted by Wylie and Anderson (43), PGC migration from the hindgut toward the gonadal ridges occurs over a period that does not exceed two days, involves roughly 400-500 cells and takes place over a terrain that is rapidly changing due to the formation of the hindgut mesentery. The first PGCs to leave the hindgut have only a few cell diameters to travel while those leaving last have considerably further to go. In any case, whatever the relative importance of the various proposed migratory mechanisms, the role of adhesion molecules remains central for this process. The presence of several adhesion molecules in PGCs, including members of all the major families of adhesion molecules, indicates that the surface of PGCs is equipped with an array of molecules allowing them to adapt themselves to the rapidly changing environment in which they develop. Nevertheless, not all PGCs successfully reach the final destination. How many fail and what is the fate of those which are lost? Do they simply die? The unique differentiation totipotency of PGCs makes possible alternative differentiation patterns. An understanding of how genes encoding adhesive molecules are regulated in PGCs and the molecular pathways associated with the functions of adhesion receptors is crucial in furthering our knowledge of PGC biology. Adhesion molecules might once again turn out to be crucial in controlling not only the germ cell lineage and PGC migration but also the PGC differentiation fate itself.

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