# CELL TECHNOLOGIES IN BIOLOGY AND MEDICINE

# Cell Biology of Fetal Tissues and Fundamental Medicine

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During the recent decade the interests of specialists in experimental mammalian embryology were focused on studies of signal networks, regulatory programs, phenotype and behavior of embryonic and mesenchymal stem cells in culture, which triggered more active use of pluripotent stem cells in cell technologies. Laboratory preparation of primary induction tissue (extraembryonic endoderm, mesoderm, hypoblast, nodule, notochord, primary streak) remains impossible, but primary and secondary organizers and antagonist tissues are essential for simulation of axial development. Only in these sophisticated heterogeneous cell system will it be possible to simulate the real physiology and bioinformatics of the embryonic leaflets, their staged transformation into definitive organs. At present, mammalian and human fetal tissues remain the carriers of unique information of the middle and late embryogenesis. For this reason, functional and therapeutic activities of fetal cells and tissues are intensely studied in foreign countries; numerous banks of fetal somatic and stem cells are created. Banks of extraembryonic umbilical blood tissue have been created in Russia. However, the biological and information potentials of fetal/embryonic tissue remain not realized at the theoretical and applied levels.

**Key Words:** *fetal tissues; stem cells; embryogenesis*

Mammalian embryogenesis is most effectively regulated by the three main powers: embryonic induction, reversible epithelial-mesenchymal conversions of cells of the embryonic leaflets and extraembryonic (EE) tissues, and embryonic stem cells (SC), their proliferation, migration, and active or dormant functional status [1-3]. Clonal expansion of regional SC plays the key role in the axial neurogenesis, somitogenesis, cardiogenesis, growth of the fetal retina and skeletal muscles [4,56,81]. Epigenomic events at the level of stem niches determine active or dormant status of SC [7,52].

The mechanism of embryonic induction starts working from the stage of primitive streak and gastrula. Axial primordium and 3D-patterning of the embryo are realized with the use of EE embryonic tissues. The organizer tissues serve not only as sources of activator signals, but also of suppressor signals, that is, the inductor tissues trigger the development of target tissues, suppressing alternative pathways of development [100].

Embryonic and fetal tissues are characterized by a special type of cell automatism: epithelial mesenchymal plasticity [2,36]. Multiple morphological status of cells is determined by a special set of genes and proteins. Reversible epithelial-mesenchymal transitions by an order of magnitude potentiate communication interactions between the neighboring tissues, modulate local counts, polarity, mobility, and location of epithelial and mesenchymal SC in provisory and definitive tissues.

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The following basic stages of embryogenesis/organogenesis are still poorly simulated or reproduced in culture of embryonic (ESC) or mesenchymal (MSC) SC *in vitro*:

- formation of the main EE provisory tissues;
- formation of epiblast from the embryoblast;
- formation of primitive streak from the epiblast;
- formation of neural plate and neural tube from the epiblast;
- formation of neural crest and its derivatives *in vitro*;
- formation of 3D-organized ecto-, meso-, and endoderm in culture;
- development of the notochord from a nodule;
- appearance of a nodule;
- appearance of anterior visceral endoderm inducing the formation of neural plate from the epiblast;
- modeling of primitive endoderm inducing the formation of hematogenic clones from the epiblast;
- modeling of cardiogenic mesoderm inducing the formation of liver primordium in the anterior intestine;
- modeling of mesoderm in epiblast culture developing after addition of the visceral mesoderm explants;
- coculturing of the mesoderm and endoderm inducing the formation of endothelium and clones of hematogenic SC *in vitro*;
- coculturing of the anterior part of epiblast and endoderm generating the endothelium and primitive hematogenic cells;
- the septum transversum endothelium stimulating the growth of the primordial liver primary epithelium into the mesenchyma (organized endothelial growth determines organized growth of liver trabeculae);
- nodule inducing axial asymmetry of the organs;
- hypoblast blocking the induction of primitive streak from the epiblast.

At present, fetal tissues are the only source of unique information about mammalian and human embryogenesis.

Here we analyze preliminary results of ESC and MSC embryogenesis *in vitro*. Analysis shows that the modern level and quality of basic knowledge, skills, technologies for obtaining laboratory tissues/implants from ESC and MSC are insufficient for basic biology and applied medicine. Creation of a long-term state program with guaranteed high technological efficiency requires a higher level of knowledge, a different scale and quality of laboratory data on the basic regularities of early embryogenesis, formation of embryonic and EE tissue. In order to understand the regularities of organized axial organogenesis, it is necessary to know how to obtain ontogenetically integral epithelial stromal tissue. This knowledge can be a result of only intense cellular genetic and morphofunctional studies of fetal and embryonic tissues. The significance of fetal tissue studies within the framework of scientific programs of research and practical application of SC is discussed [22,70,77].

## EMBRYONIC INDUCTION

In the early 1990s Spemann and Manhold showed that fragments (explants) of the mesoderm and endoderm can trigger ectopic gastrulation and even formation of the secondary developmental axis in amphibian embryos. The organizer tissues duplicating the ectopic gastrula and axial development were soon described for lower and higher animals, including mammals. In the 1930-40s, K. Waddington studied in detail the induction mechanisms of primitive streak development in chicken [95]. In the 1930-60s, the organizer tissues initiating new primordia from signal centers responsible for further axial patterning of structures were identified [5,40,65].

In mammals, EE endoderm, EE ectoderm, nodule, and the notochord are the main sources of activator signals and suppressor signals, suppressing the alternative variants of development. It was revealed in the late 1990s that the final 3D-project of an embryo is a result of multiple interactions between provisory EE tissues and ecto-, meso-, and endoderm. The concept of embryonic induction in mammals was formulated in the 1970s [12,23]. In mammals, the primitive and parietal endoderm forms from the embryoblast, while the EE ectoderm appears from the trophectoderm. Polar trophectoderm is a precursor of EE ectoderm and chorionic ectoderm. The upper layer of the embryoblast is reorganized into a primitive endoderm (hypoblast) forming a dividing basal membrane between the hypoblast and embryoblast. Later the hypoblast cells are differentiated into visceral endoderm, while cells adhering to the trophectoderm differentiate into parietal endoderm (Table 1).

The EE endoderm and EE ectoderm virtually do not form in a culture of postmitotic ESC aggregations. Because of this, the expression of the key genes of the primitive streak, mesoderm, and gastrula is significantly reduced. In parallel, differentiated cardiomyocytes, neurons, myocytes, and osteoblasts block the expression of genes working in EE provisory tissues [11,34]. In many cases, the efforts are aimed at the creation of a feeder (an inductor modeling signals from EE tissues) [110].

## LABORATORY EMBRYOGENESIS OF ESC

Any spontaneous differentiation of ESC aggregations in culture eventuates in developmental abnormalities because of anticipating differentiation of cardiomyocytes, neurons, and other somatic lines. Genetic programs of definitive somatic cells block the formation of EE tissue in accordance with the law of gene antagonism during development. Axial development, patterning, activation of Hox genes in embryos are blocked without organizer tissues [1,11,31].

In contrast to isolated ESC and MSC, the development of epiblast and gastrula primarily depends on provisory EE tissues. The functions of the hypoblast (primary endoderm) at the stage of late blastocyst are aimed at minimization of the ESC activity in the epiblast. The Dkk1 antagonists released by the hypoblast block the Wnt3a—TGFB signal cascade. The second echelon of antagonists (Cer1, Lefty1,2, Hexs1) suppresses activity of the Nodal/Cripto cascade. The ectoderm signals are neutralized by Chordin/Noggin proteins during this period. The dormant status of the epiblast is attained due to predominance of the suppressor signals over activator signals. The intrinsic Wnt3a/Nodal level in the epiblast is insufficient for induction of the primitive streak (PS). The Wnt3a/Nodal excess produces a nodule. Gastrulation starts from partial epithelial-mesenchymal transformation of the middle and posterior epiblast cells.

Mutant mouse Cert1-/-Lefty1-/- embryos form multiple fragments of ectopic PS [75]. Removal of the hypoblast or local addition of Goosecoid mRNA also causes the formation of PS ecto-copies [15]. Expression of PS and mesoderm genes automatically blocks the expression of neuroectoderm genes [28].

The first portion of mesenchymal cells of PS (10,000-20,000 cells) is spent for the formation of EE mesoderm (allantois, amnion, yolk sac), the initial part of the next PS portion (20,000-30,000 cells) is spent for the formation of cardiogenic, cranial, para-axial, and axial mesoderm. The middle and partially posterior parts of PS generate the mesoendoderm and definitive endoderm. The anterior monolayer of the epiblast is reorganized into the neural plate and tube.

## SPONTANEOUS DIFFERENTIATION OF EMBRYOID BODIES

Cultured ESC aggregations of 25-100 cells were placed onto gelatin in a serum-free medium containing no LIF. During the first 48 h, the cells rapidly adhered to gelatin and formed a monolayer of immature cells. Global restructuring of the expression of 35,000 genes took place during 2 weeks after removal of cell proliferation stimulants. The functions of more than 50% genes (pluripotency, self-reneval, stem capacity, ectoderm, provisory organs) were completely reduced, while genes of the mesoderm and endoderm gradually activated [37]. These shifts in gene expression explain why $Oct4^+$ pluripotent ESC cannot be isolated from the gastrula and postgastrula structures.

Cavitation is the second bright phenomenon of spontaneous differentiation of embryoid bodies. It manifests in massive formation of epithelial cysts (microcavities) in compact clusters of ESC. As a laboratory "caricature", cavitation simulates the formation of an amniotic cavity of postimplantation embryos. About 50% cysts are lined with primitive endoderm (hypoblast). The formation of primitive endoderm is associated with Nanog repression (endo-

**TABLE 1.** Main Induction Effects of Organizer Tissues

| Organizer tissue | Main effect |
|---|---|
| Nodule asymmetry | Induces extracopies of the nerve axis, ectoduplication of primordial limb, visceral |
| Anterior visceral endoderm, anterior epiblast | Inductors of nerve axis extracopies |
| Anterior visceral endoderm | Main organizer of embryonic head part. Induces axial patterning of neural tube, keeps the epiblast/primitive streak borderline via Wnt- and Nodal antagonists |
| Notochord (a) | Induces patterning of nerve tube and para-axial mesoderm |
| Notochord (b) | Inductor of dorsal pancreas and aorta, cardiomesoderm blocker |
| Primitive endoderm | Maintains the dormant status of ESC in epiblast and blocks the formation of primitive streak |
| EE ectoderm | Induces formation of primary germinative lines through BMP-4. BMP-8 through BMP-4 blocks the neuroectoderm |
| Mesoderm | Blocks neuroectoderm |

derm suppressor gene) [34]. Other cysts are lined with visceral endoderm and mesothelium. Active capillarogenesis takes place in the mesenchyma between epithelial cysts [44]. The capacity of polycystic embryoid bodies to generate ectopic tumors is attributed to multiple imbalances in embryonic induction [92].

In a dense culture of embryoid bodies *in vitro,* only uncoordinated formation of minifragments of the epiblast, hypoblast, and EE endoderm from E-cadherin$^+$ cells was observed [13]. In order to copy a normal gastrula *in vitro*, 650 epiblast cells should be cultured on the luminal surface of an artificial planar matrix (basal membrane). The contralateral surface of the basal membrane should be populated by hypoblast cells. Normal 3D-gastrulation and formation of mesoderm and mesoendoderm *in vitro* can be observed in a modeled epiblast/hypoblast bilayer separated by the basal membrane. The formation of PS cells in ESC aggregations in suspension *in vitro* can be evaluated by the ratio of E-cadherin$^-$/N-cadherin$^+$ cells labeled with Mixl1 mRNA [64]. Wnt3a (100 ng/ml) or activin A (25 ng/ml) best of all induces the formation of Mixl1$^+$ cells in serum-free medium. The cells of the anterior PS (Brachyury$^+$CD4$^+$FoxA2 high) and posterior PS (Barchyury$^+$CD4$^+$FoxA2 low) were obtained separately in aggregations of mouse ESC [31]. Anterior PS cells additionally express the goosecoid/cerebrus mRNA. The posterior part of PS produces Hoxb1 and Tbx6. SB431542, a selective antagonist of Nodal/TGFB/activin A signal cascade and Dkk1 (Wnt3a blocker) completely blocked the appearance of PS cells in ESC culture. Cultured PS cells during cuturing are characterized by pronounced phenotypic instability. Stable PS strains were never obtained yet.

Signal interaction between the epiblast, hypoblast, EE ectoderm, EE endoderm, and the nodule in the embryo is an obligatory condition for the formation of 3D-axial gastrula. The formation of stable provisory postgastrula structures is impossible without this signal network. Aggregations of ESC without provisory induction tissues start preventive formation of cardiomyocytes, neurons, and angioblasts without organogenesis and expression of the axial patterning genes.

## MESO-, ENDO-, AND ECTODERM FROM ESC

The first laboratory mesoderm was obtained in a culture of ESC aggregates by adding 15% fetal calf serum (FCS) [66]. However, the newly formed mesodermal cells are unstable in serum-containing medium and rapidly differentiate into somatic mesodermal cells. In serum-free medium, 1% DMSO (SRF-RhoA inductor) induces the formation of 5-8% mesodermal cells.

A combination of SRF and RhoA (cytoskeleton reorganization initiator signals) causes the formation of the first echelon of the mesoderm in the form of Brachyury$^+$Flk1$^+$PDGFRA$^+$ cells. The same primary mesoderm strains can be obtained in serum-free medium using a combination of alternative signals: Nodal, BMP-4, TGFB, or by adding BMP-4/activin A. The yield of mesodermal cell is 30-40% [32,41,91]. The yield of mesoderm from an ESC monolayer can be increased to almost 100% by coculturing with cardiogenic mesoderm [102].

A combination of BMP-4, activin A, and TGFB induces almost 50% level of Brachyury$^+$Flk1$^+$SCL$^+$ progenitors with subsequent rapid expression of GATA-1 and other hematoangiopoiesis markers. This simple ESC system simulates the key events taking place in the yolk sac [71]. In parallel, BMP-4 blocks the appearance of the neuroectoderm [74]. The bulk of new cells is directed (by the gene antagonism) to anticipating development of EE hemopoiesis.

The formation of the mesoderm from epiblast in culture was induced by adding the anterior visceral endoderm. Definitive endoderm, ectoderm, and mesoderm are unable to induce the mesoderm *in vitro* [14]. Primitive ectoderm is the strongest antagonist of the mesoderm formation. The endoderm Goosecoid/FoxA2 genes rank second by incidence.

The formation of the primitive ectoderm was observed only at low density of ESC. Selective accumulation of clonogenic nerve SC was attained using bFGF, EGF in N2 medium. Addition of the incubation medium from visceral endoderm (or medium from HEPG2 cells) was sufficient for massive induction of Sox1$^+$Hes1$^+$Hes5$^+$ cells. The BMP-2/BMP-4 blockers stimulated the neuroinduction [94,80]. Primary ectoderm was obtained as Sox1$^+$ nestin$^+$ cells from the epiblast in low density culture after addition of BMP-4 antagonists [45,99]. The maximum yield of these early cells was obtained from Cripto-/- embryos, in which the mesoderm formation was blocked.

Combination of IHH (Indian hedgehog) and bFGF signals induces the formation of angiohematogenic clones from unorganized PS mesenchyma, but not from an epiblast monolayer [16,24].

High density of cell-cell contacts in dense ESC culture and addition of bFGF/HGF induced the formation of primitive/visceral endoderm without components of basal membrane. Laminine, tanascine, type IV collagen were inessential for the formation of endodermal cells [87,108], while the formation of the epiblast and primary ectoderm (polarized

compact high epithelium) depended primarily on the matrix.

Activin A (100 ng/ml) caused rapid (7-10 days) highly effective (80%) differentiation of ESC into endoderm in serum-free medium. The differentiation consisted of 3 stages. After 24 h E-cadherin$^+$ epithelial ESC cells transformed into N-cadherin$^+$ mesenchyma. Incubation of mesenchymal cells in medium with 100 ng/ml activin A and 2% FCS led to expression of the endodermal key genes (FoxA2, Sox17, Cas, CXCR4) [19]. Expression of FoxA2 restored the E-cadherin$^+$ epithelial status of the endodermal cells. Addition of BMP-2 during this phase accelerated the formation of EE endoderm and blocked the formation of neuroectoderm [8,94]. Ras is the second inductor signal protein of EE endoderm, suppressing the expression of Nanog [104].

## ISOLATION OF EMBRYONIC LEAFLETS FROM MUTANT EMBRYOS

The ESC isolated from mutant SRF-/- embryos differentiate predominantly into the endoderm in a monolayer culture. The absence of SRF (a potent mesoderm inductor) automatically directs the development of pluripotent stem cells into the endoderm [97]. *In situ* the Nodal-Cripto is the most potent inductor for the formation of the mesoderm and cardiomesoderm. Early Cripto-/- mouse embryos consist of disorderly growing nerve tissue. The Cripto-/- ESC aggregations never generate mesoderm and cardiomesoderm derivatives [73,103]. The Bmi-/- mouse embryos contain a 30-50-fold excess of neural SC in the neural tube in comparison with common control animals [60]. Endodermal cells were obtained in a culture of embryoid bodies by blocking the expression of Oct4 by means of selective iRNA [35]. Mutant EED-/- embryos produce a mesoderm excess at the expense of the ectoderm and neuroectoderm deficiency [61]. These abnormal embryos can serve as primary sources for isolation and accumulation of immature clonogenic pluripotent tissue. Other variants of abnormal formation of embryonic and EE tissue are known (Table 2).

## EMBRYONIC INDUCTION IN PRIMORDIAL HEART AND LIVER

Cardiac morphogenesis starts from the appearance of two symmetrical condensations of precardiogenic mesoderm (heart fields) located at the interface with the parietal endoderm. During the same time the nodule releases Dkk1 selective inhibitor of the Wnt signal system into the adjacent endoderm. Then Dkk1 triggers the expression of Hex in the parietal endoderm and participates in the organ specification of the mesoderm [29].

The first cardiomyocytes appear from splanchopleura through the Sparc inductor, released by the parietal endoderm. Then Sparc triggers the expression of the cardiomyocyte key genes (GATA-4, GATA-6, Nkx 2.5, BMP-2, Mef2C) [88]. Cardiogenic mesoderm, in turn, secretes hepatogenic inductors (aFGF, bFGF, FGF8, BMP-2/BMP-4), which modulate the primitive endoderm of the primary intestine [90]. The indispensable role of the parietal endoderm in cardiogenesis was confirmed in a culture of mouse ESC: embryoid bodies spontaneously differentiated in the presence of a feeder from fetal embryoblasts, the percentage of resultant cardiomyocytes being retained at the level of 7-16%. Coculturing of the same ESC aggregations with the parietal endoderm explant increased the percentage of spontaneously contracting cardiomyocytes to 95-100% [84].

After formation of the cardial loop the atria and ventricles grew at the expense of directed expansion of 140-180 founder clones [25]. The signals and mechanisms of directed growth of the clones are not studied. The proepicardium (coronary artery precursor) is also a part of the splanchopleura mesothelium. Another part of the mesothelial plast migrates to the future primordial liver.

The primordial liver appears from the primary intestinal definitive epithelium in the septum transversum area (mesoderm surrounding the middle intestine). The definitive endoderm exists in the postgastrula for a short period. In contrast to ESC, the definitive endoderm explants poorly proliferate

**TABLE 2.** Embryonic Tissue Abnormalities

| Gene defect type | Main type of morphosis |
|---|---|
| BMP-2+/+ | Hyperproduction of EE endoderm. No neuroectoderm |
| FGF-8-/- | Gastrula without mesenchyma |
| BMP-1RA-/-, β-catenin-/-, Smad-4 -/- | Gastrula without mesoderm |
| Nodal-/- | No primitive streak, EE endoderm; neuroectoderm excess |
| Ras1-/- | No EE endoderm |
| SHH-/- | No axial skeleton and lungs |

and retain the immature phenotype in culture. However, the primitive endoderm undergoes axial regional differentiation into the esophageal, gastric, small intestinal, and colorectal epithelium under the effect of regional mesenchyma [93].

The neighboring cardiogenic mesoderm, releasing BMP-4 and bFGF, serves as the direct inductor of primordial liver [82]. These signals trigger the expression of GATA-4, GATA-6, FoxA2, and Hex [106,107]. Hex (euchromatin corepressor) blocks preterm differentiation of the definitive endoderm [46].

The liver stroma appears from migrating mesodermal splanchopleural progenitors and contains much endothelium and endothelial progenitors. The vasculogenic stroma directs organized growth of the epithelial primordial liver. Chaotic growth of hepatoblasts without formation of lobules/trabeculae was observed in flk-1-/- embryos. Interactions between endothelial progenitors and hepatic progenitors were studied in culture. Incubation of the primary intestinal epithelial monolayer and septum transversum mesenchyma leads to rapid organized growth of endothelial progenitors and endodermal cells into the primordial mesenchyma. Direct contact of the endodermal cells and endothelium causes induction of Hex, α-fetoprotein mRNA synthesis. The presence of the endothelium is an obligatory condition for the endoderm growth into the mesenchyma and production of Hex, Prox-1, and α-fetoprotein mRNA [57]. The flk-1-/- embryos died in utero on days 9.5-10.5 of development because of multiple defects of the yolk and embryonic circulation. However, the isolated 10-day flk-1+/- primordial liver rapidly grows in culture. During 72 h the flk-1+/- primordial liver increases its cell content 15-fold. It is noteworthy that up to 20% hepatoblasts appear in the flk-1+/- explant. Less than 5% hepatoblasts were found in the flk-1-/- explant, which confirms the direct involvement of the primordial liver endothelium in hepatogenesis [9].

In parallel, the future liver endoderm induces the development of cardiogenic mesoderm [110]. The first hepatoblasts appear in mouse primordial liver on day 14.5 of development. Clones realizing the future three dimensional design of the liver appear during this period. The brain, heart, small intestinal and lung epithelium, retina, and skeletal muscles form by dispersion of the stem clones [1,4]. These clone-forming cells were isolated from fetal liver (13.5 days of development) by flow cytofluorometry. The clones were obtained on a laminin (or type IV collagen) underlayer. Liver SC coexpressed CD29 and CD49f (integrin β1- and α6-subunits). By their phenomarkers the isolated cells differed from the typical oval cells. Solitary adhesive cells produced homogeneous colonies of 80-100 cells within 5-7 days. Study of the heterogeneity of the resultant colonies showed that c-met$^+$, CD49f low, c-kit, CD45, and Ter119 cells formed the bulk of the clones. The cells did not express MHC 1 on their surface. The resultant clones remained heterogeneous by size and cell counts. Clones producing albumin or cytokeratin 19 or both were identified in the culture. Typical oval CD34$^+$thy-1$^+$ cells appeared in the colonies on days 19-21 of culturing [18,69,76].

Hepatocytes form from ESC aggregations (embryoid bodies) in serum-free medium in the presence of three inductors: BMP-4, activin A, and bFGF. The life span of definitive endoderm and the percentage of endodermal cells in the culture are negligible. This confirms the hypothesis about the existence of alternative hepatogenesis from MSC and ESC [33].

## PRIMARY AND SECONDARY INDUCTORS OF NEUROGENESIS AND ORGANOGENESIS

The anterior EE endoderm and the nodule release follistatin, Noggin, and chordin (BMP-4/BMP-2 antagonists). Blocking of BMP signals leads to the formation of the head neural tube by the "default" mechanism. The formation of the tail neural tube requires other inductors (retinoic acid, FGF, Wnt3a) [85]. Any organizer is a heterogeneous population of cells with a varying set of signals, different trajectory of development, and life span. The mechanism of organizer tissue formation is not deciphered. Studies of the cellular and signal interactions in the organizer are just beginning. It was found that the Goosecoid expression stimulates the formation of the head part of the neural tube, blocking the "tail neurogenesis" at the level of Wnt8 expression blocking [101].

Fetal and embryonic tissues remain the only source for simulation and studies of the middle embryogenesis *in vitro*. The head neural tube is subjected to segmentation into prosomers (forebrain), mesomers (midbrain), and rhombomers (metencephalon). The segmented structures demonstrate clonal expression of nerve SC clones and serve as the source of secondary signals for the appearance of neuroglial lines [38].

After the gastrula is over, the external surface of the embryo is covered by a monolayer of primitive ectoderm. The area without FGF signaling transforms into the primary epidermis under the effect of BMP-4/BMP-2 excess. The epidermis area is selected due to activation of the Wnt cascade,

which reduces the activity of FGF receptors [30]. The formation of multi-lamellar epidermis is controled by BMP-4/BMP-2 and Notch. However, the underlying mesenchyma in the zone of epithelial placode formation produces BMP and FGF antagonists.

The placode epithelium responds by production of EGF and Wnt. The primordial hair bulb appears under the effect of a combination of negative/positive signals. Activation of the classical Wnt-β-catenin signal cascade in the majority of cases promotes the formation of multilamellar epithelial placode. Secretion of SHH from the epithelium into the mesenchyma causes the formation of mesenchymal condensate (papilla) under the placode. The set of FGF, Wnt, BMP, and a series of other accessory signals limits the placode area governed by the mesenchymal inductive signals. New primordial teeth, hair, or mammary (salivary) gland can be derived from any fetal epithelium by its coculturing with competent mesenchyma. Studies aimed at derivation of primordial teeth, hair, and sensory organs using chimerical fetal epithelium and mesenchyma under laboratory conditions are now in progress [68,79,89,96].

Unique, little studied programs for the formation of the eye, teeth, sensory organs are loaded in mammalian and human fetal tissues; the realization of these programs starts in the middle of somitogenesis. Regional fetal mesenchyma or epithelium (but not local SC) are in its epicenter. Study of the genetic and epigenetic bases of the fetal placode and papilla competence is of great practical significance. Cellular mechanisms of the primordium and development of teeth, mammary and salivary glands, hair follicles, and sense organs are characterized by universal structure with a universal set of genes. Primordial placode progresses into polarization of the epithelium and formation of a multilamellar primordium. The epithelial structure is then subjected to invagination into the mesenchyma and branched tubulogenesis [17].

The visceral endoderm induces (via IHH) the formation of the primitive ectoderm in aggregations of mouse ESC. The main marker of this provisory tissue is complete absence of the mesoendoderm key genes in the presence of the minimum positive expression of FGF5, Rex-1, Gbx2. Coculturing of embryoid bodies together with the first neuroectodermal cells on a poly-d-lysine matrix in DMEM/F12/N2 led to the appearance of morphologically immature progenitors expressing nestin, Sox-1, and Sox-2. This population symmetrically proliferates. The second discriminative feature of early embryonic neuroprogenitors is excessive expression of the neural tube axial assembly genes (Pax2, Pax6, Mash1, Math4a, Delta1, Islet1) [51].

In contrast to isolated ESC and neural SC, axial development of the neural tube is associated with organized proliferation/migration of neural SC clones. This programmed behavior of cells is regulated by special signals of EE embryonic tissue [53-56,83].

The organizer located in the cerebral isthmus plays an important role in axial regionalization of the head part of the neural tube. Ectopic transplantations of the isthmus explants into the neural tube cause the formation of the rhombomer extra copies [6]. These "secondary organizers" predetermine the species-specific characteristics of the cerebral morphogenesis [1].

Only in fetal tissue culture is it possible to reproduce:

- signal antagonism of ecto-, meso-, and endoderm;
- cooperation/antagonism of embryonic tissues and provisory extraembryonic inductor tissues and inhibitor tissues;
- branching morphogenesis and the secondary organizer effects;
- appearance of epithelial placode and mesenchymal papilla;
- characteristic cicatrix-free regeneration of fetal tissue;
- formation of dormant SC (including the dormant stem niches);
- reversible epithelial-mesenchymal conversion of cells in embryonic tissues and organs;
- appearance of primitive SC in the heart, brain, liver, limbus, skeletal muscles, and other organs;
- appearance of dormant tumor cells in the bone marrow;
- formation of cell strains from somites, neural tube, placode, and neural crest (local inductor/suppressors).

## THE MESONEPHROS: AORTOGONADAL PRIMORDIUM

This provisional organ of the dorsal aorta functions as an intermediate hematogenic cell donor for fetal hemopoiesis and generates a unique $CD34^+Flk1^+c\text{-}met^+$ pluripotent angioblast population, providing anticipating formation of vessels in all emerging organs of the fetus. Moreover, the dorsal aorta angioblasts serve as the primary source of the dormant mesodermal progenitors, emerging in all adult organs [59].

In parallel with this, the aortogonadal primordium is an important intermediate depot of myo-

blasts. The density of desmin$^+$MyoD$^+$ cells in this organ is higher than in somites [20]. The myogenic precursors migrate from here to all skeletal muscles and form the regional SC reserves. The signal characteristics of this primordium are virtually not studied. Banks of these cells are created for practical use.

## PRIMARY VASCULOHEMATOPOIESIS IN THE YOLK SAC

The first vascular endothelium appears at the interface between the mesoderm and endoderm. The contact of EE embryonic endoderm with the somatopleural mesoderm (not a precursor of hematogenic tissue) leads to the formation of hematogenic clones and endothelium [72]. The primitive endoderm induces the formation of endothelium and hematogenic tissue in the explant of the anterior part of mouse epiblast [14]. The first vascular network of the yolk sac appears as a result of "signal roll call" between the EE mesoderm and EE visceral endoderm. The visceral endoderm secretes IHH, which induces the formation of BMP-4 in EE mesoderm. In turn, BMP-4 induces the expression of vasculogenesis key genes (Flk1, CD34, VF-cadherin). IHH and FGF first trigger the formation of angioblasts from the PS mesenchyma (mesoderm). Further expression of hemangioblast common genes (flk-1, CD34, SCL, flt-4) is regulated by IHH, BMP-4, bFGF. The identity of arterial endothelium of macro- and microvessels, similarly as the identity of venous endothelium, is established before the beginning of circulation [16,24].

## BANKS OF FETAL TISSUES

Introduction of fetal tissues in basic and applied medicine started from creation of federal and private cell banks [26]. Banks of hematogenic stem cells from fetal liver were created in the mid 1990s in Europe and the USA for the treatment of hereditary anemias, immunodeficiency, and malignant hematological diseases in children [4,21,27,39, 43,48-50,58,63,78,98]. The unique material accumulated by today is used for the creation of cell culture library (including EE embryonic tissues), verification of new mechanisms of development detected in laboratory mammals. Attempts at collecting blood cells and tissues from donors with hereditary diseases are made.

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