Review

Signaling Control of Differentiation of Embryonic Stem Cells toward Mesendoderm

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Abstract

Mesendoderm (ME) refers to the primitive streak in mammalian embryos, which has the ability to further differentiate into mesoderm and endoderm. A better understanding on the regulatory networks of ME differentiation of embryonic stem (ES) cells would provide important insights on early embryo patterning and a possible guidance for ES applications in regenerative medicine. Studies on developmental biology and embryology have offered a great deal of knowledge about key signaling pathways involved in primitive streak formation. Recently, various chemically defined recipes have been formulated to induce differentiation of ES cells toward ME *in vitro*, which greatly facilitate the elucidation of the regulatory mechanisms of different signals involved in ME specification. Among the extrinsic signals, transforming growth factor-β/Activin signaling and Wnt signaling have been shown to be the most critical ones. On another side, intrinsic epigenetic regulation has been indicated to be important in ME determination. In this review, we summarize the current understanding on the extrinsic and intrinsic regulations of ES cells-to-ME differentiation and the crosstalk among them, aiming to get a general overview on ME specification and primitive streak formation.

Introduction

One of the most important events in embryogenesis is the generation of three germ layers: ectoderm, mesoderm and endoderm during gastrulation of embryo development. In mouse, once the oocyte is fertilized with a sperm, it forms a zygote, which progressively develops into morula, a mass of cells (blastomeres), after a series of cleavage divisions. Along with the formation of a cavity inside the morula, the embryo develops to blastocyst, with the outer cells becoming trophectoderm and the inner cells forming the inner cell mass (ICM). The ICM is composed of the pluripotent cells and the blastocyst interior cells. The pluripotent cells further become epiblast that is the source of three germ layers of gastrula, while the blastocyst interior cells will form the primitive endoderm, which give rise to the endoderm layer of extraembryonic tissues. Then gastrulation starts, which is marked by the formation of a transient structure called primitive streak in the region of the epiblast; during this process, uncommitted epiblast cells mobilize, egress to form the primitive streak then quickly exit from the primitive streak and form the mesoderm or defined endoderm [1]. The mesoderm gives rise to bone, heart, vascular tissue, muscle and kidney while the endoderm develops to lung, liver, pancreas, stomach and intestine (Fig. 1a).

The ICM-derived mouse embryonic stem (ES) cells and epiblast-derived human ES cells have the full potential to differentiate to ectoderm, mesoderm, endoderm and trophoblast. During their differentiation to mesoderm and endoderm, these ES cells go through an intermediate stage called mesendoderm (ME), which is equivalent to the primitive streak [2–4]. However, different from human ES cells that can be directly induced to differentiate to ME, mouse ES cells need to be induced to form embryoid body before ME differentiation [5,6].

Several genes have been shown to specifically express in the primitive streak and to be required for primitive streak formation (Table 1). For instance, *Brachyury* (*T*), which is expressed throughout the primitive streak [7], and loss-of-function studies indicate its essential role in primitive streak formation and ME differentiation [8]. As a T-box transcription

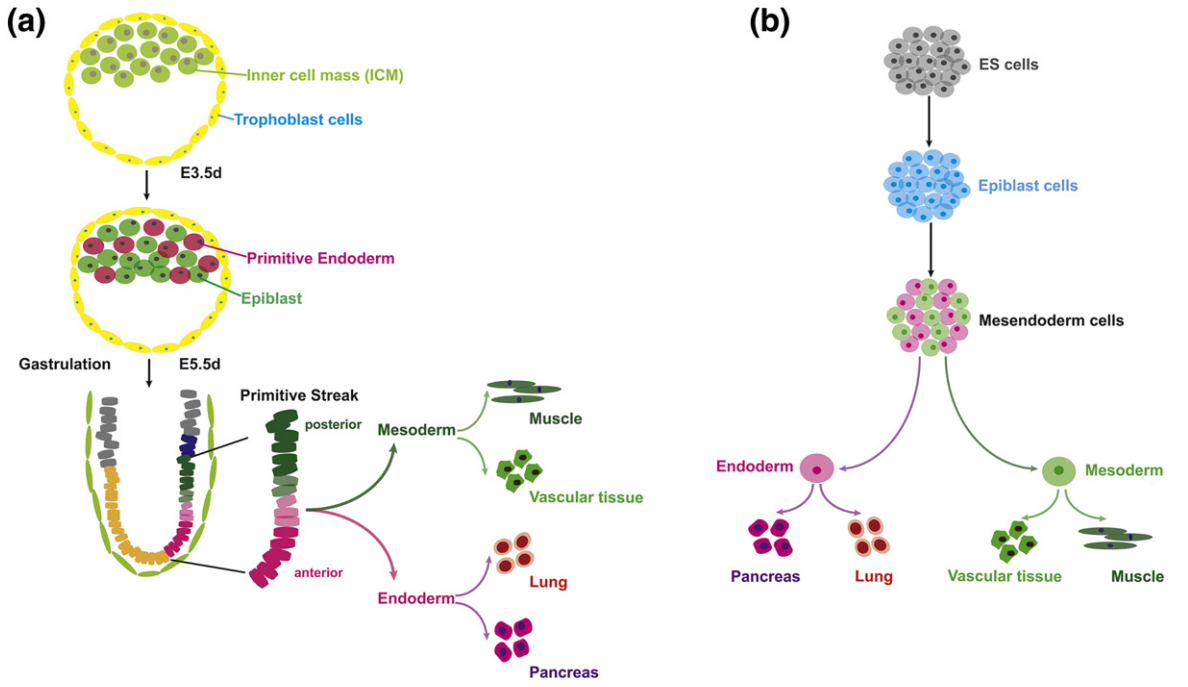

Fig. 1. Primitive streak formation during mouse embryogenesis and ME differentiation of ES cells. (a) When a mouse embryo develops to about embryonic day 3.5, it forms a blastocyst, containing the outer blastomeres that are the precursors of trophoblast cells, and the inner blastomeres that form ICM. The ICM is further differentiated into pluripotent epiblast cells and the cells that contribute to the primitive endoderm. At about E5.5 day, gastrulation begins: epiblast cells form a transient structure-primitive streak. The anterior part of the primitive streak eventually develops endoderm tissues such as pancreas, lung and liver, while the posterior part forms mesoderm tissues such as bone, vascular tissue and muscle. (b) ES cells derived from the ICM have the potential to form ectoderm, mesoderm, endoderm and trophoblast. Along with the differentiation, ES cells become epiblast cells, which have the capability to differentiate to all layers except the trophoblast. Epiblast cells can further differentiate to ME, which is equivalent to the primitive streak, and subsequent mesoderm and endoderm tissues.

factor, *Brachyury* controls the expression of many developmental related genes. Similarly, the *Mix-like homeodomain protein 1 (MixL1*) is also expressed throughout the primitive streak, and its ablation results in failure of primitive streak formation and ME differentiation [9]. However, the expression of *MixL1* seems to be delayed and is only detected in later stages of differentiation. In addition, there are

Table 1. Signature genes of primitive streak/ME

Gene	Expression		Loss-of-function effect		Reference
	ES cells differentiation	Mouse development	ES cells differentiation	Mouse development	
Brachyury (*T*)	24–48 h	Primitive streak	Fail to differentiate into ME	Failure of gastrulation and primitive streak formation	[8]
MixL1	24–48 h	Primitive streak	Fail to differentiate into ME	Abnormalities in primitive streak and node formation	[9]
Eomes	24–48 h	Posterior primitive streak	Fail to differentiate into ME	Failure of EMT and loss of primitive streak	[13]
Wnt3	24–48 h	Primitive streak	Fail to differentiate into ME	Inhibition of gastrulation and loss of primitive streak	[11] and [12]
Nodal	~24 h	Epiblast/gastrulation/ primitive streak	Fail to differentiate into ME	Arrest at egg cylinder and fail to form primitive streak	[13]
Fgf8	48–72 h	Prestreak- and streak-stage embryos	—	Failure of gastrulation and primitive streak formation	[10]
FoxA2	48–72 h	Anterior primitive streak	—	Normal gastrulation and primitive streak formation	[142]
Goosecoid	48–72 h	Anterior primitive streak	—	Normal gastrulation and primitive streak formation	[143]