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Review: Genetic manipulation of the rodent placenta

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ABSTRACT

The principal role of the placenta is the maintenance of pregnancy and promotion of fetal growth and viability. The use of transgenic rodents has greatly enhanced our understanding of placental development and function. However, embryonic lethality is often a confounding variable in determining whether a genetic modification adversely affected placental development. In these cases, it is beneficial to specifically manipulate the placental genome. The purpose of this review is to summarize available methodologies for specific genetic modification of the rodent placenta. By restricting genetic alterations to the trophoblast lineage, it is possible to gain a deeper understanding of placental development that perhaps will lead to gene-targeted therapies to rescue irregular placentation in transgenic animals or in women at high-risk for placenta-associated pregnancy complications.

1. Introduction

The placenta functions to facilitate the exchange of molecules between maternal and fetal circulations and to provide endocrinological and immunological support for pregnancy. Aberrant placental development is implicated in many pregnancy complications, impacting both fetal development and maternal health [1]. Therefore, gaining a deeper understanding of placental development, both normal and irregular, is of significant clinical importance.

The placenta contains anatomically distinct zones wherein architecture and function is dependent on the progressive differentiation of trophoblast cells. Trophoblast cells comprise a heterogeneous population of cells derived from progenitor trophoblast stem (TS) cells [2]. Trophectoderm, the eventual source of TS cells, is the first lineage-committed cell-type to appear during embryogenesis, emerging as polarized cells segregated to the periphery of the blastocyst. The remaining internal cells form an inner cell mass from which two populations of lineage-committed cells have been derived: extraembryonic endoderm (XEN) and embryonic stem (ES) cells [3]. While XEN and ES cells are fated to form yolk sac endoderm and all germ layers of the fetus, respectively, TS cells undergo a multilineage differentiation pathway resulting in distinct subpopulations of specialized trophoblast cells. Characterization of these trophoblast subtypes has revealed unique ontogeny, morphology, and function [4].

In rodents, the placenta can be divided into two regions: the junctional zone and the labyrinth zone. The junctional zone is an active endocrinological and invasive tissue situated at the uterine-trophoblastic border, and is comprised of trophoblast giant cells, spongiotrophoblast cells, and glycogen cells. Later in gestation, the invasive trophoblast lineage develops within the junctional zone, and actively migrates into the maternal decidua [5]. The labyrinth zone is the site at which transfer of nutrients between maternal and fetal circulations occurs, and is comprised of mesenchymal villous structures bordered by fused syncytiotrophoblast and a discontinuous cytotrophoblast layer bathing in maternal blood. Although some aspects of placentation differ between humans and rodents, there are fundamental similarities between these species in terms of placental structure, development, and function [6–8]. Thus, the capacity to genetically modify rodent trophoblast subpopulations, or the placenta in general, provides valuable information on processes integral for placental development and function.

The purpose of this review is to discuss available methodologies for genetic modification of the rodent placenta. Whole-body gene manipulation has enabled researchers to identify roles for genes in many tissues; however placental function is inherently vital for fetal viability, thus embryonic lethality is often a confounding variable in deciphering whether a genetic modification affected placental or fetal development. By restricting the genetic manipulation to the placenta, it is possible to more clearly define the effects on placental development and function. Techniques for genetic modification of the placenta, which are represented schematically in Fig. 1, will be categorized as follows: i) transgene expression using trophoblast-specific promoters; ii) cell-based modulation of

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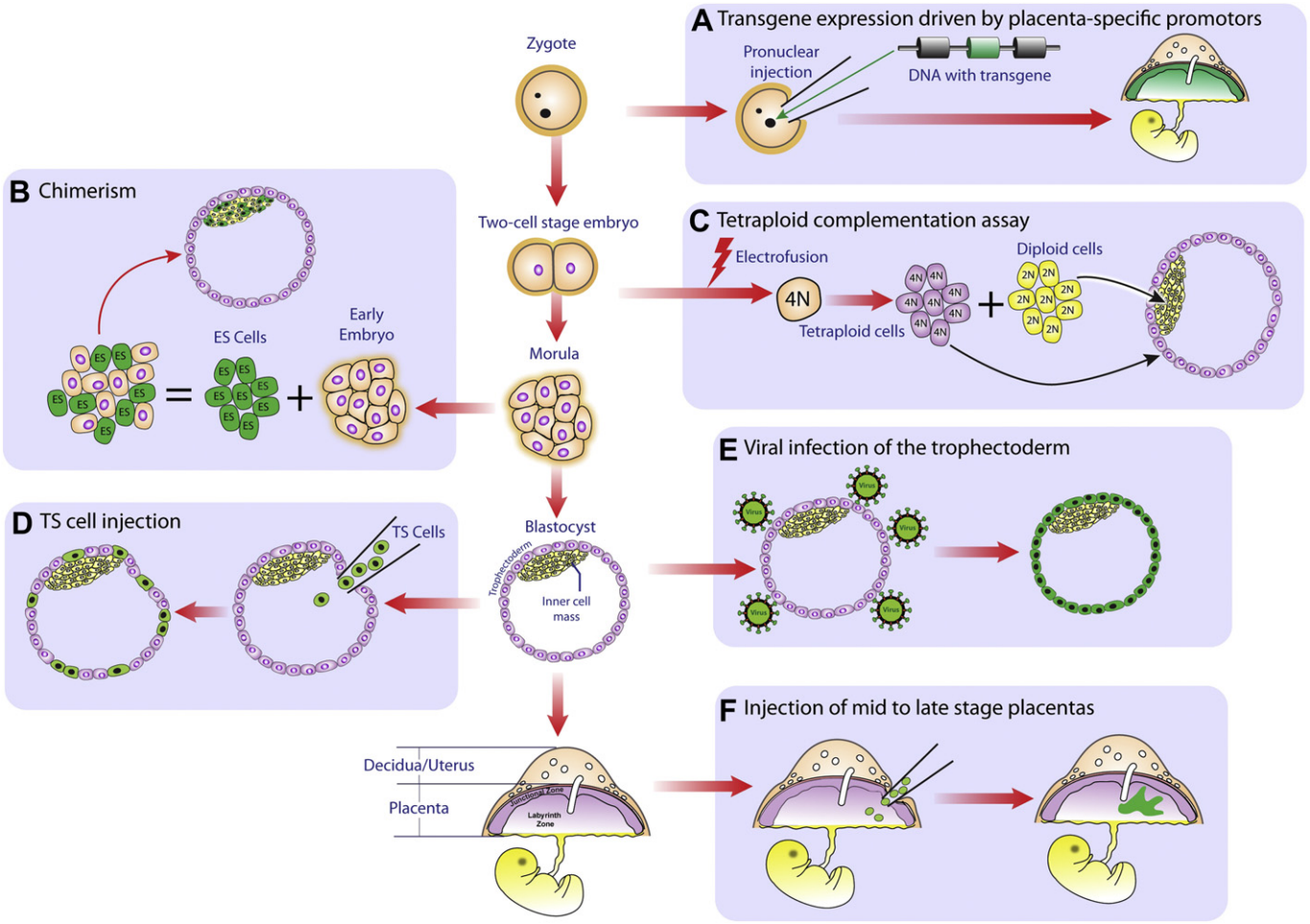

Fig. 1. Schematic representation of techniques to facilitate genetic modification of the rodent placenta. Unboxed flow chart in middle of diagram depicts progression from zygote through to mature conceptus. A) Pronuclear injection of transgene-containing DNA driven by placenta-specific promoter. Note that only the mature placenta expresses the transgene (green). B) Generating a chimera by aggregating an early embryo and transgenic ES cells. Please observe that ES cells are only capable of contributing to the inner cell mass. C) Tetraploid complementation. Electrofusion of a two-cell stage embryo results in the generation of a single tetraploid cell. Tetraploid cells continue to proliferate and develop normally up until the blastocyst stage, but rarely contribute to embryonic structures. Therefore, aggregation of tetraploid and diploid embryos results in lineage exclusivity, with tetraploid cells wholly contributing to extraembryonic tissue. D) Injection of TS cells into the blastocoel. Injected TS cells contribute exclusively to the trophectoderm. E) Viral infection of a blastocyst results in transduction of the peripheral trophectoderm, whereas inner cells are shielded from viral exposure. F) Intra-placental engraftment of cells or injection of virus to mature placentas, resulting in regions of placenta that express foreign cellular proteins or are transduced with virus, respectively.

placental gene expression, and iii) viral strategies for genetic manipulation of the placenta.

2. Trophoblast-specific gene promoter mediated manipulations

Genetic modulation of the placenta eliminates the ambiguity associated with embryonic lethality in whole-body transgenics. This approach is made feasible by insertion of genes downstream of promoters that are active in specific trophoblast subpopulations. Inserted genes are then expressed only in trophoblast subtypes containing the active promoters, where they facilitate gain-of-function or loss-of-function effects. The critical limitation for this strategy is the identification of gene promoters that are active with relative exclusivity in trophoblast cell-types. Thus far, several such genes have been recognized (Table 1). In some cases, such as with the purine-catabolizing enzyme *adenine deaminase (ADA)*, the gene is active in all trophoblast derivatives [9]. Trophoblast *ADA* expression, which commences at mid-gestation and increases until term, protects the embryo from purine cytotoxicity. Using transgenic mice, it was found that a 770-bp region situated 5.4-kb upstream of the *ADA* transcription start site is sufficient to drive expression of a reporter construct exclusively in the mouse placenta [10]. Therefore, transgene insertion downstream of the *ADA* promoter may be a putative means of expressing transgenes in all trophoblast derivatives.

Many genes exhibit a temporal or cell-specific expression pattern in the placenta and may be useful for targeting trophoblast subpopulations during certain gestational periods. Such is the case with the rodent prolactin (PRL) superfamily. The genes that encode these proteins are organized contiguously on the same chromosome. Each gene shows a characteristic temporal and trophoblast cell-specific expression pattern [11]. Although the promoter motifs that drive *in vivo* expression of most PRL family members are unknown, the endogenous *placental lactogen-II* (*PL-II; Prl3b1*) promoter, which is activated in rodent trophoblast giant cells and, at least in mice, spongiotrophoblast cells during the latter half of pregnancy, has been effectively used to target gene expression to the mouse placenta [12,13]. In particular, a region spanning −2019 to −1340 bp upstream of the *PL-II*