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Human cytotrophoblasts acquire aneuploidies as they differentiate to an invasive phenotype

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Abstract

Through an unusual differentiation process, human trophoblast progenitors (cytotrophoblasts) give rise to tumor-like cells that invade the uterus. By an unknown mechanism, invasive cytotrophoblasts exhibit permanent cell cycle withdrawal. Here, we report molecular cytogenetic data showing that ~20 to 60% of these interphase cells had acquired aneusomies involving chromosomes X, Y, or 16. The incidence positively correlated with gestational age and differentiation to an invasive phenotype. Scoring 12 chromosomes in flow-sorted cytotrophoblasts showed that more than 95% of the cells were hyperdiploid. Thus, aneuploidy appears to be an important component of normal placentation, perhaps limiting the proliferative and invasive potential of cytotrophoblasts within the uterus.

Keywords: Placenta; Cytotrophoblast; Aneuploidy; Proliferation; Differentiation; Invasion; Fluorescence in situ hybridization

Introduction

Development of eutherian mammals depends on the placenta. Formation of this transient but vital organ presents an interesting opportunity to study seemingly unique processes. For example, uterine attachment and acquisition of a maternal blood supply require the organ's specialized epithelial cells (i.e., cytotrophoblasts [CTBs]) to aggressively invade maternal tissues (Fisher et al., 1989; Librach et al., 1991). Except for the fact that CTB invasion is limited to the decidualized endometrium and the inner third of the myometrium, this process is more akin to tumorigenesis than to organogenesis.

What is the origin of the CTB subpopulation with tumor-like properties? These cells arise by differentiation of a progenitor population that is anchored to the trophoblast basement membrane surrounding the mesenchymal cores of chorionic villi. In one pathway, CTBs leave this basement membrane and fuse to form a continuous layer of syncytiotrophoblasts (STBs) that cover the chorionic villi (Fig. 1). These floating villi, so named because they float in maternal blood, are the site of hormone production as well as nutrient, gas, and waste exchange between the mother and the fetus. In the second pathway, the focus of this study, CTBs at the tips of anchoring chorionic villi leave the trophoblast basement membrane and form columns of nonpolarized cells that attach to and then penetrate the uterine wall. The ends of the columns terminate within the superficial decidua, where they give rise to invasive CTBs (Fig. 1). During interstitial invasion, a subset of these cells, either individually or in small clusters, commingles with resident decidual, myometrial, and immune cells. During

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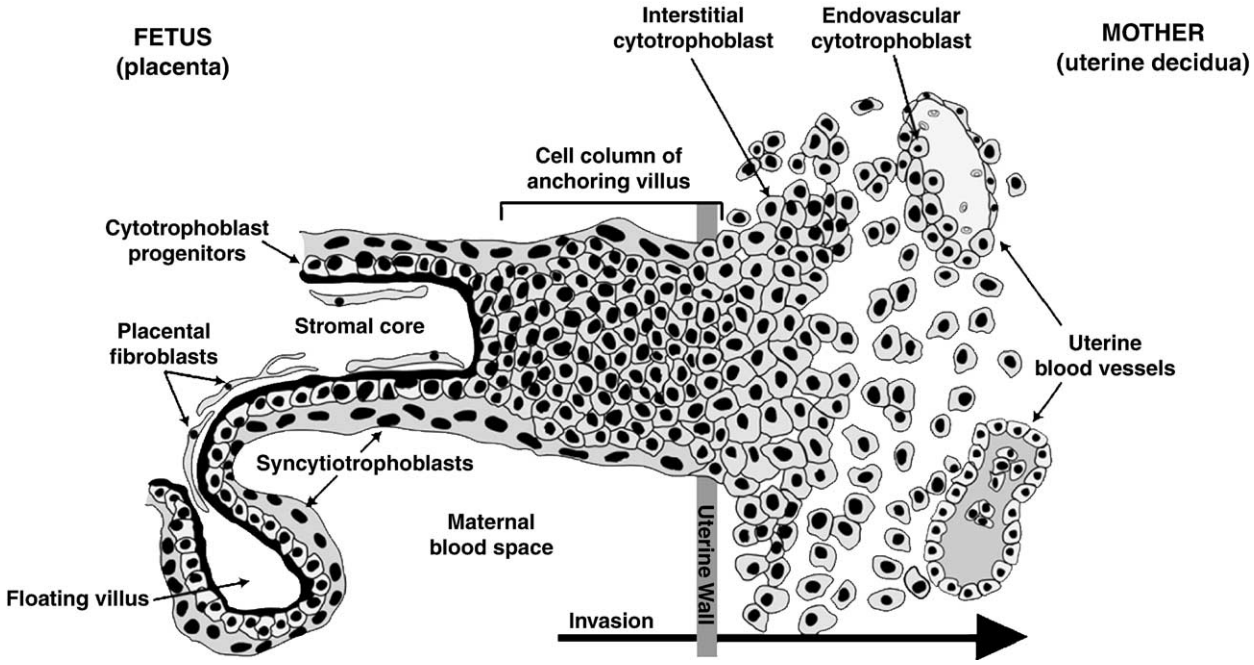

Fig. 1. Diagram of the maternal–fetal interface at about 10 weeks of gestation. The floating villi, which are suspended in the intervillous space, are bathed by maternal blood. The anchoring villi, which function as a bridge between the fetal and maternal compartments, form cell columns that give rise to the subpopulation of cytotrophoblasts (CTBs) that invade the uterine interstitium (decidua and first third of the myometrium, interstitial CTBs) and maternal vasculature (endovascular CTBs), thereby anchoring the fetus to the mother and accessing the maternal circulation.

endovascular invasion, masses of CTBs migrate into the vessels before the lumina eventually recanalize (Brosens et al., 2002; Zhou et al., 1997). Together, these two components of CTB invasion anchor the placenta to the uterus and divert uterine blood flow to the intervillous space.

There are several critical transitions in CTB differentiation along the invasive pathway that are marked by the cells' expression of stage-specific antigens (Damsky and Fisher, 1998). Before differentiation, the CTB progenitor subpopulation, which is attached to the trophoblast basement membrane, is actively proliferating; many of these cells stain positively for Ki67, an antigen expressed throughout the active phases of the mitotic cell cycle (Schwarting, 1993). CTB expression of this antigen is abruptly downregulated as the cells invade the uterus, thus uncoupling invasion from proliferation (Genbacev et al., 1997). Additionally, the cells upregulate expression of molecules that mediate invasion (e.g., matrix metalloproteinase-9) as well as immune regulators (e.g., HLA-G, a class Ib major histocompatibility complex molecule). Although the mechanisms are not yet fully understood, HLA-G is thought to play an important role in maternal tolerance of the hemiallogeneic CTB subpopulation that invades the uterine wall, i.e., the cells to which expression of this antigen is restricted (McMaster et al., 1995).

With regard to the genetic aspects of placental development, chromosomal abnormalities–the major cause of spontaneous abortions–are surprisingly common (Hassold and Jacobs, 1984). Cytogenetic studies of human preimplantation embryos have shown that mitotic errors such as nondisjunction or anaphase lag occur frequently. In some patients, at least 50% of in vitro fertilized embryos show evidence of aneuploidy and chromosomal mosaicism (Munné, 2002). Generally, about one third of all embryos are lost in the first trimester, with the majority of cases occurring between implantation and recognition of pregnancy (Miller et al., 1980; Wilcox et al., 1988). Thus, it appears that abnormal embryos are selected against in the first few weeks of life by mechanisms that involve failures in critical elements of implantation and placental development.

In most pregnancies, the fetus and the placenta have the same karyotype because both lineages are descendants of the same zygote. However, in ~1 to 2% of pregnancies, chorionic villus sampling (CVS) reveals confined placental mosaicism (CPM) in which the placenta and the fetus have different karyotypes (Kalousek and Vekemans, 1996; Ledbetter et al., 1992). CPM can occur as a result of postzygotic errors in mitosis, in which case the conceptus may have a normal karyotype. Alternatively, a trisomic blastocyst may be rescued by chromosome loss within the embryo, leaving the extraembryonic lineages trisomic.

To date, many genetic studies have examined floating villi and the cells they contain, including the trophoblast populations. Very little is known about the karyotype of human CTBs that arise from anchoring villi and subsequently invade the uterine wall. Interestingly, in mice, the analogous population of invasive trophoblasts undergoes endoreduplication (MacAuley et al., 1998; Nakayama et al., 1998). A few reports suggest the possibility that invasive human CTBs have an elevated ploidy level (hypertetraploid