CLINICAL IMPLICATIONS OF BASIC RESEARCH

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The Placenta — Fast, Loose, and in Control

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The placenta never fails to amaze. The conventions that we take for granted as required for the normal formation and function of other organs are often flouted by placental trophoblasts, the specialized epithelial cells that carry out the myriad functions of the placenta. In a recently published genomic analysis of 86 placentas, Coorens et al.[1] provide yet another example of a flouted convention.

Through genome sequencing of chorionic villous tissue at term or its component microdissected compartments (multinucleated trophoblasts and mesenchyme), the authors found that individual trophoblast clusters in every sample had a mutational burden similar to that found in pediatric cancers. Furthermore, clonal copy-number variants were evident. In another twist, different regions of each placenta were genetically distinct. The high rate of somatic mutations and regional specificity enabled phylogenetic reconstruction of the mutational origins, which in some cases could be traced back to the zygote. Monoclonal outgrowths of trophoblasts were physically enormous, occupying a majority of each biopsy specimen and forming a patchwork of spatially confined outgrowths that must have arisen early in placental development. No major variation from the clonal mutation patterns associated with normal pregnancy was observed in placenta-driven complications. Thus, the authors conclude that clonal genetic mosaicism is a normal feature of the placenta (Fig. 1). The placental cells differ from those of the normal colon or endometrium in having much larger monoclonal expansions. Cancer driver mutations were not detected.

The mechanisms behind these findings are as yet unknown. A clue to the origin of the copy-number variants is their coincidence with fragile sites on the chromosomes. Presumably, these breaks do not elicit the DNA-damage response that ordinarily results in cessation of cell proliferation. The somatic mutations in each clonal outgrowth bore the signature of oxidative DNA damage, possibly reflecting the fact that implantation and the early stages of placental development take place in a physiologically hypoxic environment, in which the percentage of oxygen is estimated to be 2%, before the placenta accesses a consistent supply of maternal blood at the end of the first trimester. Although not examined in this study, another factor could be the unusual nature of the placental and trophoblastic epigenome. The epigenome represents a temporally dynamic pattern of chemical modifications to DNA that "cross-talk" with post-translational modifications of the histone proteins around which DNA is wrapped. The pattern determines, in part, which genes are expressed and guides the surveillance of and response to DNA damage. Epigenetic modifications are also critical for chromosomal stability.

As compared with the epigenomes of most other cell types, the placental epigenome is notably nonconforming in that it is hypomethylated, and in this respect it resembles the epigenomes of cancer cells.[2,3] Although most alterations in cancer cells and in the placenta are "passengers" of no consequence, cancers have several genetic driver mutations that change epigenetic marks and confer uncontrolled proliferation. These observations raise new questions as to how the cancerlike epigenome of placental cells and its physiologic environment intersect in the acquisition of the clonal genetic alterations described by Coorens et al.

The results of this study have interesting implications for preimplantation genetic testing

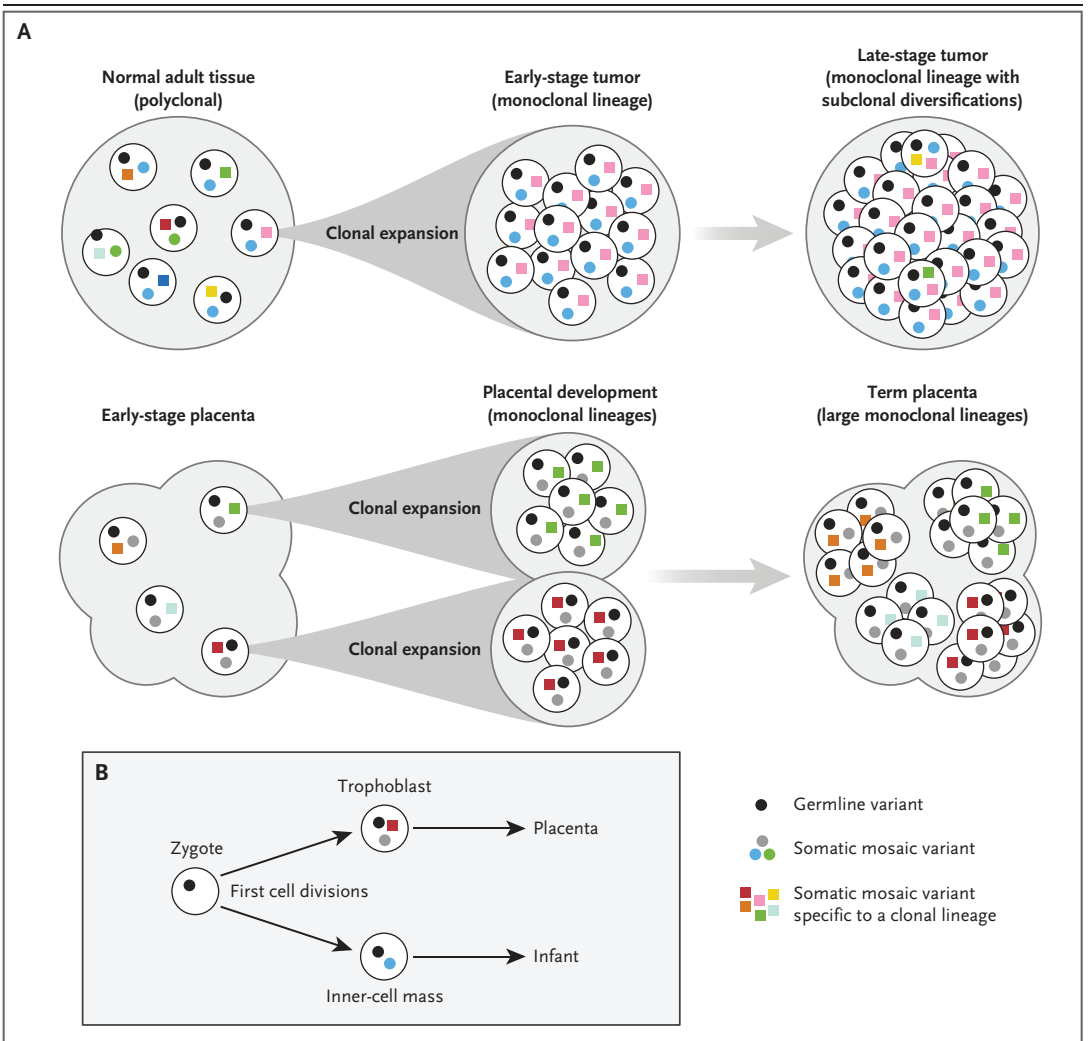

Figure 1. Accrual of Somatic Variants in Context.

A bulk piece of normal human tissue from an adult contains many tiny clonal expansions (Panel A) — some with cancer driver mutations — that are detectable only with very sensitive methods. Coorens et al. recently described large monoclonal lineages — easily detectable on whole-genome sequencing of specimens obtained from placental biopsies — with a mutation burden similar to that found in tumors in children but lacking in cancer driver mutations. Accordingly, every placental-biopsy specimen that the researchers examined had both mutations that were shared with the other specimens and mutations that were specific to that specimen (so-called private mutations) and were not present in the fetal tissues (Panel B). The earliest opportunity for the segregation of cells of placental lineages from those of fetal lineage is within the first few cell divisions of the embryo. In some cases, the data described by Coorens et al. suggest that this segregation may have been nearly complete.

and prenatal diagnosis of aneuploidies. Currently, trophoblast biopsy is performed routinely to obtain embryonic cells for analysis.[4] Although problems related to the technologies used to analyze small numbers of cells contribute to misdiagnoses, the work of Coorens et al. offers an additional explanation. The authors found segregation of the trophoblast and inner-cell mass lineages (which develop into the placenta and embryo, respectively) within a few cell divisions of fertilization and describe a case in which this bottleneck rescued the inner-cell mass from trisomy of chromosome 10. Similarly, these genetic bottlenecks could explain the rela-

tively high rate of confined placental mosaicism (aneuploidy detected only in portions of the placenta) that is estimated to affect approximately 2% of placentas. Going forward, as more sensitive sequencing methods are developed and applied to prenatal diagnosis, clinicians should anticipate finding higher rates of the types of mutations and copy-number changes the investigators describe. In this regard, it will be important to understand the range of "normal" and "abnormal" genomic changes in terms of pregnancy outcomes, which won't be an easy task. The same issues will probably affect prenatal diagnoses made with the application of advanced sequencing methods to the analysis of free fetal DNA in maternal blood and biopsy specimens of chorionic villi.

Coorens et al. profiled only a subset of trophoblasts, namely, the multinucleated cells that form the surface of the placenta, termed syncytiotrophoblasts (STBs). Cytotrophoblasts (CTBs), which fuse to form multinucleated STBs, have yet to be investigated. Presumably, the mutational load and copy-number variants that CTBs carry are highly related to those of STBs. However, these progenitors can differentiate into a different subpopulation of trophoblasts — extravillous CTBs. As their name implies, these cells leave the chorionic villi that make up the placenta. In a cancerlike process, they deeply invade the uterus and remodel the resident spiral arteries into large-bore, low-resistance vessels that carry substantial amounts of maternal blood to the placenta. Previously, fluorescence in situ hybridization was used to show that both CTB progenitors and their extravillous progeny include substantial numbers of aneuploid cells at the whole-chromosome level.[5] It will be important to advance the current work to include these unusual cells.

The findings reported by Coorens et al. may also have implications for pregnancy complications associated with abnormal placentation, including intrauterine growth restriction, preeclampsia, and a subset of preterm births. In this regard, the authors analyzed bulk placental tissue from 21 pregnancies in which there was extreme growth restriction, with or without preeclampsia, but did not detect differences in the type or load of mutations as compared with pregnancies with normal outcomes. Still, many interesting questions remain. Extreme growth restriction and preeclampsia have a common feature: defects in the extent to which extravillous CTBs invade the maternal vasculature. Uterine invasion is shallow, and vascular transformation is incomplete, factors that reduce maternal blood flow to the placenta. In the presence of these complications, does the mutational burden of CTBs differ from that observed in similar cells from normal pregnancies? Also, the authors describe the frequent detection, in placental genomes, of chromosome breaks affecting vulnerable areas that typically accrue mutations with age. Might copy-number break points in other fragile regions occur in malfunctioning placentas? Could these cumulative genetic defects in the absence of cancer drivers help to explain the 9-month lifespan of the placenta? The work of Coorens et al. has opened the door to answering these and many more questions of clinical importance.

Disclosure forms provided by the authors are available at NEJM.org.

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