Medical Xpress

Placental RNA may help protect embryo from viruses, study finds

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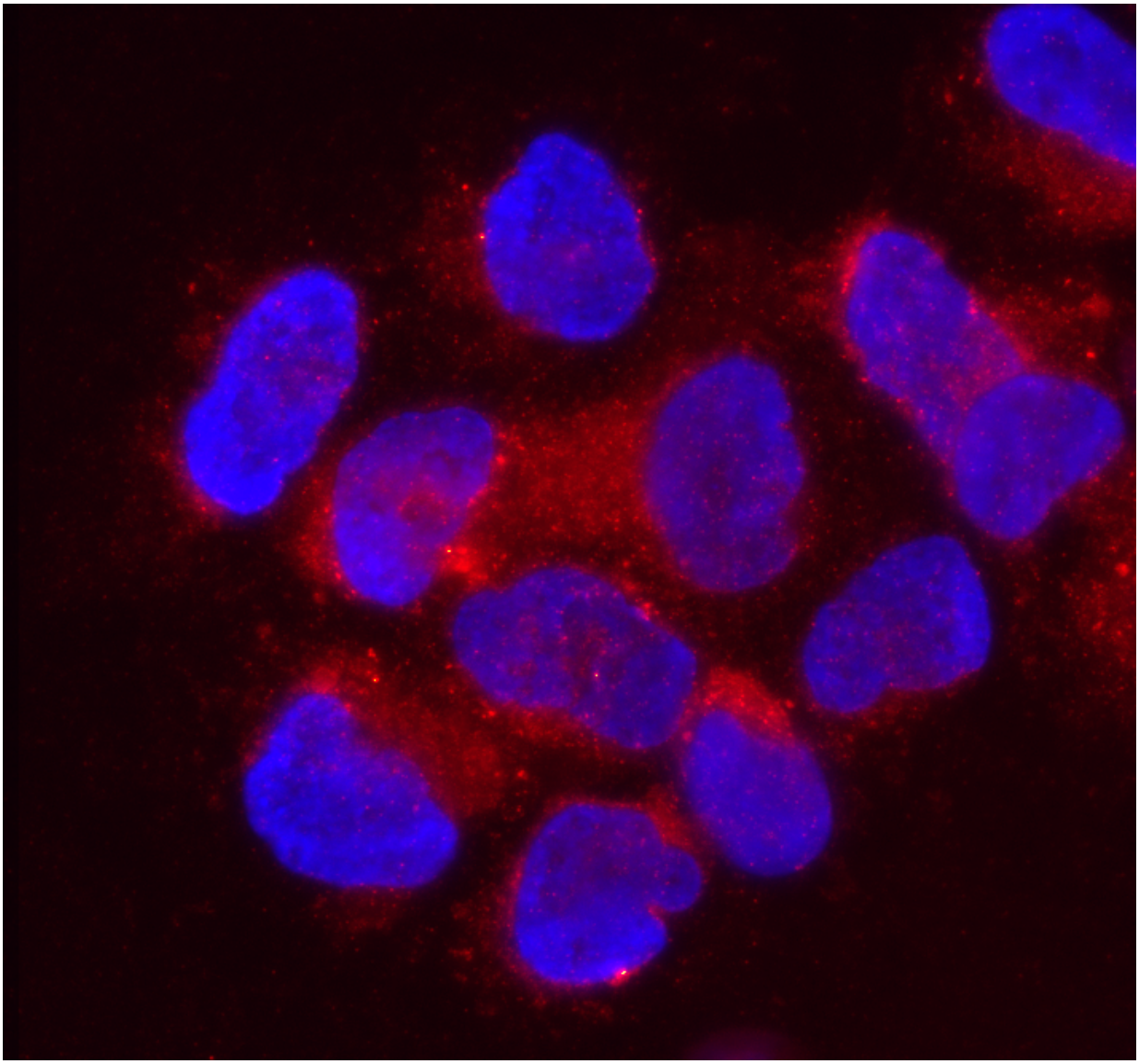

A new University of Pennsylvania study found that placental cells are rich with

lncRHOXF1 (red), which appears to offer the developing embryo protection from viruses. Credit: University of Pennsylvania

The human placenta is an organ unlike any other. During the course of nine months it is formed by the embryo, sustains life and then is shed.

"What that means," said Montserrat Anguera, an assistant professor in the University of Pennsylvania School of Veterinary Medicine, "is it has to make very specialized cells, it has to form structures to support itself and the baby, it has to sense cues from the mom and from the environment and it has to do all of these things really, really fast."

In a new study, Anguera and colleagues have identified a long non-coding RNA, or lncRNA, that contributes to a crucial function of the placenta: protecting the unborn baby from invading pathogens.

The work, published in the journal *Molecular and Cellular Biology*, is the first to identify a lncRNA in the placenta involved in regulating the immune response.

Further study of this and other lncRNAs could shed light on how the placenta protects against pathogens, even at the earliest stage of embryotic development. Long-term, the researchers say, it's possible that this lncRNA could even present a target for priming the placenta to resist viruses or other infectious agents.

Anguera collaborated on the work with lead author Ian Penkala, Jianle Wang, Camille M. Syrett and Carolina B. López of Penn Vet and Laura Goetzl of Temple University.

Knowledge about lncRNAs is fast-evolving, and it's an area that Anguera

has been part of since her time as a postdoctoral researcher. As their name suggests, lncRNAs are RNA transcripts greater than 200 nucleotides in length that do not code for proteins. Many of them are known to regulate gene expression and to do so in a rapid manner.

Other researchers have identified lncRNAs in later-term placentas involved in regulating functions such as growth, but Anguera wanted to look at the earliest stages of placental formation to see how lncRNAs might be influencing development.

Using data from an earlier paper by a Chinese group that had sequenced RNA in various stages of early human development, Anguera's team zeroed on a lncRNA called lncRHOXF1, located on the X chromosome, that was present at high levels in trophectoderm cells, from which the placenta arises, and barely detectable in the cells that give rise to the embryo. Not only was it present in trophectoderm cells, it was one of the most abundant lncRNAs in that cell type.

They then went about characterizing lncRHOXF1. Using computer models, they confirmed that it was unlikely to code for a protein and, because it had strong matches to non-human primate genomes, as well as those of elephants and dogs but not for mice, it was likely a recently evolved lncRNA.

Using an in vitro model, they found that levels of the lncRNA were highest two days after embryotic stem cells began to differentiate, the same time point at which these cells begin to express markers that distinguish them as placental precursor cells. The researchers also confirmed the presence of the molecule in various cell types, at low levels in human first-trimester placenta and placenta cell lines and at higher levels in extravillous cytotrophoblasts, or the precursors of the portion of the placenta that implants in the maternal uterus, and at the highest levels in their in vitro system, the human embryotic stem cells.

The findings suggested that lncRHOXF1 appears to play an important role very early in placental development.

Next they determined where in the cell the lncRNA was expressed and were surprised to find it at high levels dispersed throughout the nucleus and the cytoplasm, suggesting it may regulate the expression of genes that are distant from it.

"It was all over the place," Anguera said. "That was a good thing for us in studying it, because if you can detect it easily it's more likely to have a robust phenotype that you're going to pick up on."

The subsequent experiments were designed to reveal what that phenotype, or function, was. Overexpressing it in undifferentiated human embryonic stem cells caused cells to grow more slowly and tend toward differentiation. When the team looked at how gene expression was altered with overexpression of the lncRNA, they found an influence on about 150 genes, many involved in DNA synthesis, packaging and replication, as well as metabolism.

When they repressed expression of the lncRNA in their in vitro system, they again found that the expression of many genes was altered, with a noticeable emphasis on genes involved in viral and immune responses.

The findings intrigued Anguera, and Penkala, currently a V.M.D.-Ph.D. student who presented them at a Penn Vet Student Research Day. López, a virologist, was in the audience and was likewise intrigued. She and Anguera struck up a collaboration which led to the final set of investigations of this study.

In these, the researchers took cells in which lncRHOXF1 expression had been disrupted and infected them with Sendai virus. They found that cells in which the lncRNA had been blocked expressed less viral RNA,

indicating a less severe infection. They also observed that lncRHOXF1 levels increased in these cells after viral infection.

"The lncRNA seems to be sensing and modulating its expression based on the virus being there," Anguera said. "People have found other examples of lncRNAs being important in regulating the innate immune response, but no one has looked in the placenta early in development."

Anguera and colleagues will be further studying this lncRNA to see if it is also responsive to different types of virus or perhaps even other types of pathogens. They would also like to gain a better understanding of how the presence of virus is translated into a message to increase levels of this lncRNA and to induce the corresponding changes in expression of genes involved in viral response.

"What we are really excited about is to pretreat or prime these placental cells by inhibiting lncRHOXF1 and see if they will be less resistant to viral infections," Anguera said. "Especially with so much current interest in Zika, it could be really interesting to see whether different viruses elicit the same type of response."

More information: Ian Penkala et al, LNCRHOXF1: a long noncoding RNA from the X-chromosome that suppresses viral response genes during development of the early human placenta, *Molecular and Cellular Biology* (2016). DOI: 10.1128/MCB.01098-15

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