Scientists discover why teeth form in a single row

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(PhysOrg.com) -- A system of opposing genetic forces determines why mammals develop a single row of teeth, while sharks sport several, according to a study published today in the journal *Science*. When completely understood, the genetic program described in the study may help guide efforts to re-grow missing teeth and prevent cleft palate, one of the most common birth defects.

Gene expression is the process by which information stored in genes is converted into proteins that make up the body's structures and carry its messages. As the baby's face takes shape in the womb, the development of teeth and palate are tightly controlled in space and time by gene expression. Related abnormalities result in the development of teeth outside of the normal row, missing teeth and cleft palate, and the new insights suggest ways to combat these malformations.

The current study adds an important detail to the understanding of the interplay between biochemicals that induce teeth formation, and others that restrict it, to result in the correct pattern. Specifically, researchers discovered that turning off a single gene in mice resulted in development of extra teeth, next to and inside of their first molars. While the study was in mice, past studies have shown that the involved biochemical players are active in humans as well.

"This finding was exciting because extra teeth developed from tissue that normally does not give rise to teeth," said Rulang Jiang, Ph.D., associate professor of Biomedical Genetics in the Center for Oral Biology at the University of Rochester Medical Center, and corresponding author on the *Science* paper. "It takes the concerted actions of hundreds of genes to build a tooth, so it was amazing to find that deleting one gene caused the activation of a complete tooth developmental program outside of the normal tooth row in those mice. Finding out how the extra teeth developed will reveal how nature makes a tooth from scratch, which will guide tooth regeneration research."

Why Extra Teeth Formed

When we lose our baby teeth, the permanent teeth grow in to replace them, but permanent teeth when lost are lost for good. U.S. adults aged 20 years and older are missing an average of four teeth due to gum disease, trauma or congenital defects. Tooth loss makes chewing difficult, causes speech problems, accelerates oral disease, and disfigures the face. Current treatments for missing teeth include dentures or dental implants, but each procedure comes with disadvantages. The idea of growing teeth to replace missing ones has captured the imaginations of scientists, with many labs investigating ways to regenerate teeth.

In the current study, Jiang and colleagues generated mice that lacked the oddskipped related-2 (Osr2) gene, which encodes one of many

transcription factors that turn genes on or off. "Knocking out" (deleting) the Osr2 gene resulted in cleft palate, a birth defect where the two halves of the roof of the mouth fail to join up properly, leaving a gap. Secondly, and surprisingly, the Osr2 "knockout" mice developed teeth outside of the normal tooth row. Jiang decided to focus his research first on the effect of Osr2 on teeth patterning (vs. cleft palate) because much more was known at the time about teeth development pathways.

Although teeth usually do not become visible until after birth, their formation starts early in development. Teeth develop from the epithelium and mesenchyme, two key tissue layers within the mammalian embryo. The first sign of tooth development in mammals is the thickening of the epithelium along the jaw line to form a band of cells called the dental lamina. Because all teeth subsequently form from the dental lamina, the assumption was that some special quality of epithelial cells there made them "tooth competent." Classical experiments, however, found that the developing tooth mesenchyme was capable of inducing tooth formation from epithelial tissues that normally would not participate in tooth development. Researchers confirmed that it was indeed the mesenchyme that carried tooth initiation signals later in development, but how those signals were restricted to the area beneath the tooth row was unknown.

Past studies in other labs had shown bone morphogenic protein 4 (BMP4) to be an important factor for the initiation of teeth, and that a protein called Msx1 amplifies the BMP4 tooth-generating signal. Jiang and colleagues suggested for the first time that some unknown factor was restricting the growth of teeth into one row by opposing the Bmp4 signal.

The current study provides the first solid proof that the precise space where mammals can develop teeth (the "tooth morphogenetic field") is shaped and restricted by the effect of Osr2 on the expression of the

Bmp4 gene within the mesenchymal cell layer. Jiang's team has shown not only that removing the Osr2 gene results in extra teeth outside of the normal row, but also that Osr2 is expressed in increasing concentration in the jaw mesenchyme as you move from the cheek toward the tongue in the mouse embryo, the exact opposite of the BMP4 concentration gradient. Osr2 restricts Bmp4 expression to the tooth mesenchyme under the dental lamina, and in Osr2's absence, Bmp4 gene expression expands into the jaw mesenchyme outside of the tooth row.

A second major finding of the study backs up another emerging theory which holds that careful regulation of competing pro- and anti-tooth initiation signals controls how mammalian teeth come one by one in sequence. As each tooth develops, something must prevent it from forming too close to the next or mammals would have no gaps between their teeth. When this mechanism occasionally falters, adjacent teeth come in fused together. Since evolution is not perfect, wisdom teeth (third molars) often come in too close to their predecessors, and must be pulled to make space.

Jiang and colleagues also engineered a group of mice with both the Osr2 and Msx1 genes removed. While mice without Msx1 failed to grow any teeth, mice lacking both Msx1 and Osr2 grew the first molars, but no additional teeth. Thus, without Osr2, enough BMP4 was expressed for the first molar teeth to grow, but without Msx1, the BMP4 signal was not amplified to the point where it could kick off the next tooth in the row. With these results, Jiang argues that BMP4 cooperates with other factors to create a temporary zone around each tooth where no other tooth can grow. When the tooth gets closer to maturity, Msx1 overwhelms decreasing levels of inhibitory factors to start the BMP4-driven development of the next tooth. Since the jaw is growing at the same time teeth are forming, it follows that each tooth must also receive signals that enough jaw has grown in for the next tooth to start forming atop it.

The implications of the current results may go beyond tooth development, researchers said. Thanks to the work of Jiang and others, some of the biochemical pathways involved in cleft lip/cleft palate development are now recognized, and may include BMP4, Msx1 and OSR2 as well as several others. In humans, Msx1 mutations have been linked with cleft lip/palate and with the failure to develop one or more teeth. In the next phase of the team's work, researchers will look at what other factors may be regulated by Msx1 and Osr2 to begin pinpointing the genetic network that controls teeth patterning and palate development. Their goal is to manipulate stem cells to treat malformations and to develop prevention strategies for cleft palate (e.g. the inclusion of folic acid in prenatal vitamins prevents neural tube defects in some cases). Cleft lip/palate occurs one in 700 live births.

Along with Jiang, the work was led by Zunyi Zhang and Yu Lan within the Center for Oral Biology and Department of Biomedical Genetics at the Medical Center. Yang Chai collaborated on the effort from the Center for Craniofacial Molecular Biology at University of Southern California School of Dentistry in Los Angeles. The work was sponsored by the National Institute of Dental and Craniofacial Research, part of the National Institutes of Health.

"Beyond medical applications, our results suggest that diversity in the number of tooth rows across species may be due to evolutionary changes in the control of the BMP4/Msx1 pathway," Jiang said. "In mammals, Osr2 suppresses this pathway to restrict teeth within a single row."

Source: University of Rochester Medical Center (news : web)