# Cystathionine gamma lyase and hydrogen sulfide: new players in orthodontic root resorption

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Orthodontic tooth movement is a safe, effective, and very widely used approach to ameliorate defects in dental alignment and craniofacial architecture (1). Although orthodontics is best known for its use to enhance aesthetics, it also serves an essential role in improving functionality, particularly in repairing severe defects in dentition that arise from genetic disorders, like Cleidocranial dysostosis or cleft palate, or which occur after severe trauma. Low levels of root resorption commonly occur when teeth are subjected to orthodontic force, but only rarely is sufficient resorption triggered to be clinically relevant (2). Though rare, severe root resorption during orthodontic treatment is difficult to predict or to detect. This can result in the loss of teeth that had been healthy prior to the orthodontic procedure. Understanding the risk factors for orthodontic root resorption is therefore of considerable interest to the orthodontic community.

In a recent article, Lu, Chen and Hua from Tongji University identified cystathionine gamma lyase (CSE) activity as an aggravator of orthodontic root resorption in a mouse model (3). CSE is an enzyme that is involved in the processing of homocysteine to cysteine and alpha keto butyrate (4). Homocysteine is a non-proteinogenic, sulfur-containing amino acid formed during metabolism of the essential amino acid methionine. Levels of circulating homocysteine are determined by the balance of biosynthesis and catabolism (5). Synthesis of homocysteine via transmethylation of methionine is catalyzed by the enzymes S-adenosylmethionine synthetase, methyltransferase, and S-adenosylhomocysteine hydrolase in three sequential steps. Catabolism of homocysteine can occur by two pathways, remethylation to methionine and trans-sulfuration to cysteine. The enzyme methyltetrahydrofolate reductase (MTHFR) is involved in remethylation. During transulferation homocysteine is first processed to cystathione, by cystathione-β-synthase (CAB) and then is converted to cysteine by CSE. Mutations in both MTHFR and CBS are associated with elevated circulating homocysteine levels, which are linked to cardiovascular disease (6), clots in veins, complications in pregnancy, and various other conditions including, autism (7), cognitive impairment or dementia (8), Down's syndrome, osteoporosis (9), movement disorder, migraines, multiple sclerosis, and polycystic ovary syndrome. Mutations in CSE by contrast are generally benign, but have been associated with intellectual disability (10).

In addition to playing a role in the very important process of catabolism of homocysteine, CSE also produces hydrogen sulfide ($H_2S$) as a byproduct of the enzymatic reaction it facilitates. $H_2S$ has recently emerged as a signaling molecule functioning in processes that include neuromodulation in the brain and smooth muscle relaxation (11). It can also modulate inflammation, insulin-release and angiogenesis (12). Recent studies provide evidence that $H_2S$ also has a role in controlling osteoclast activity. For example, $H_2S$ was reported to trigger osteoclast production and receptor activator of nuclear factor kappa B-ligand (RANKL) expression in rats (13), and $H_2S$ produced by CSE was shown to promote orthodontic tooth movement (14,15). The mechanism by which $H_2S$ exerts its effects

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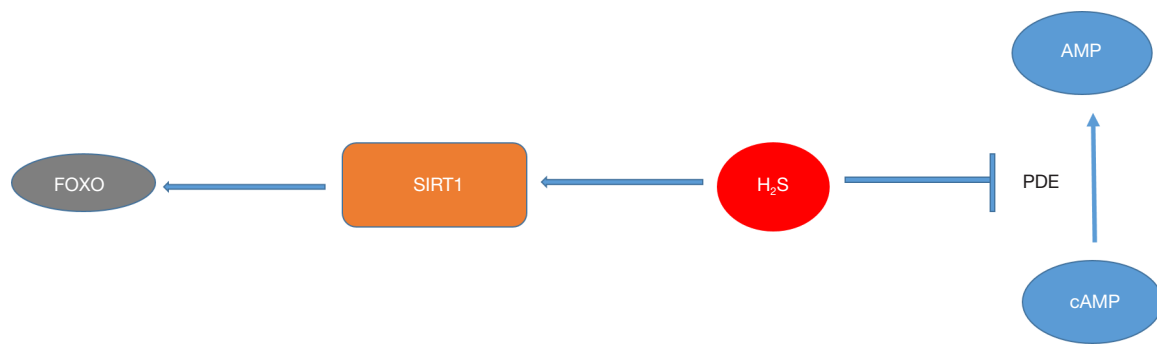


**Figure 1** Hydrogen sulfide signaling related to bone biology. Hydrogen sulfide has been shown to regulate Sirtuin1 (Sirt1), which regulates expression of Forkhead Box O (FOXO) transcription factors, and cAMP phosphodiesterase. These are important regulators of bone remodeling.

on osteoclasts and bone cells are not well characterized, but evidence suggests that it stimulates sirtuin 1 (16) and inhibits cAMP phosphodiesterase activity (17), both plausible regulators of osteoclasts and osteoblasts (*Figure 1*). It was therefore reasonable to hypothesize that CSE, and $H_2S$ produced by CSE, might have a role in orthodontic induced root resorption, a process that includes both stimulating uncoupled resorption and inflammation.

In their recent article, Lu and colleagues first showed evidence by microCT of teeth that root resorption increased with age in mice to a maximum age of 52 weeks, but that CSE knockouts had lower levels of root resorption. Root resorption also increased over a period of three weeks in wild type mice compared with no force controls, or mice in which CSE was knocked out. This data was measured first by microCT, then results were confirmed by analysis of hematoxylin and eosin stained sections. Tissues contained less RANKL and osteoprotegerin mRNA in CSE knockout mice, and the RANKL/osteoprotegerin ratio was higher in wild type mice compared with CSE knockouts. Consistent with these data, CSE mice had fewer root-associated odontoclasts. Taken together, this suggests that mice with no CSE, and presumably lower $H_2S$ levels, display fewer odontoclasts and less root resorption.

Because there are conditions in humans that can result in increases or decreases in CSE activity, there is potential immediate clinical relevance to these data. In humans, severe deficiency in CSE, leading to cystathioninuria, is most typically described as benign, but evidence for neurological defects in childhood has been presented (18). Excess of $H_2S$ from CSE activity can be the result of hyperhomocysteinemia (19). This can result from kidney disease, lack of B vitamins in the diet, hypothyroidism,

alcoholism, and certain types of medications (19). Might the rare patients who have clinically significant orthodontic-induced root resorption exhibit excess CSE activity leading to root resorption? Although there is no data that directly addresses this question, indirect evidence suggests that this is unlikely. Chronic binge drinkers, where $H_2S$ would be expected to be higher (20), display reduced orthodontic tooth movement compared with controls (21).

Can strategies to increase CSE activity or local $H_2S$ be used to increase rates of tooth movement, or could local inhibition of CSE activity and reduction in $H_2S$ be used to curtail root resorption? In Lu *et al.*, the rate of tooth movement in the CSE knockout was not reported. Based on previous research by the same authors it would presumably be slower than wild type. How much relative to root resorption is a crucial question. It is possible that root resorption is more responsive to CSE activity than bone resorption. In that case a small increase in CSE activity may have little effect on tooth movement rate, but may greatly enhance root resorption. Likewise, reductions in CSE activity may not greatly influence tooth movement, but may be associated with large decreases in root resorption. It is also possible that root resorption is less sensitive to CSE activity changes than tooth movement, in which case it can be imagined that local addition of $H_2S$ might significantly enhance tooth movement, while largely sparing the teeth from additional root resorption. Future studies carefully examining the effects of changes in CSE activity, and local $H_2S$ levels, on both tooth movement rates and root resorption will be crucial.

Although the interpretation presented in the present study is that CSE triggers root resorption through $H_2S$, this was not directly demonstrated. The enzymatic pathways associated with the metabolism of methionine

and catabolism of homocysteine are complex, and some elements, homocysteine for example, have direct effects on bone and other connective tissues that could influence root resorption. If $H_2S$ production is key, then better understanding of how it exerts effects on cells would increase our understanding of root resorption and orthodontic tooth movement. Currently three distinct mechanisms of $H_2S$ signaling have been identified: (I) reduction and/or direct binding of metalloprotein heme centers; (II) serving as a potent antioxidant through reactive oxygen species/reactive nitrogen species scavenging [probably the mechanism of induction of Sirt1 (22)]; or (III) persulfidation, the post-translational modification of proteins by addition of a thiol (-SH) group onto reactive cysteine residues (23,24). Further understanding of the role of CSE and $H_2S$ in root resorption will depend on identifying the mechanisms that support CSE's ability to stimulate bone and root resorption.

## Acknowledgments

## Footnote

*Ethical Statement*: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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