A dual role for 7-dehydrocholesterol reductase in regulating Hedgehog signalling?

In a recent paper published in *Development*, Koide et al. provide evidence for a negative regulatory action of 7-dehydrocholesterol reductase (DCHR7) on the Hedgehog pathway (Koide et al., 2006). In a series of elegant experiments, the authors show that: (1) DHCR7 expression is intimately linked to the expression of Sonic Hedgehog (Shh); (2) DHCR7 functions as a negative regulator of Shh signalling in various model systems; (3) the reductase activity of DHCR7 is indispensable for DCHR7's inhibitory action, whereas the N terminus seems to be essential in this respect; and (4) DHCR7 acts at the level of Smoothened (Smo).

These findings surprised us at first, as we recently published that mouse patched 1 (Ptch1) secretes vitamin D3, which subsequently binds to and inhibits Smo (Bijlsma et al., 2006). A consequence of our findings is that a defect in (or the absence of) DHCR7 that leads to the accumulation of its substrate, 7-dehydrocholesterol (7-DHC, the precursor of vitamin D3), should inhibit Smo activity (through enhanced Ptch1-dependent vitamin D3 secretion). Indeed, we have shown that the DHCR7 inhibitor AY-9944 or the genetic absence of DHRC7 [using $Dhcr7^{-/-}$ cells (Cooper et al., 2003)] results in the increased repression of Smo. By contrast, however, the experiments performed by Koide et al. indicate that knocking down or pharmacologically inhibiting DHCR7 has a stimulatory effect on the Hedgehog pathway rather than an inhibitory one, indicating that DHCR7 acts as a negative regulator of the Hedgehog pathway. Thus, these data apparently conflict with our work, and also with earlier papers that suggest a positive regulatory role for DHCR7 on the Hedgehog pathway. Obviously these paradoxical results raise questions.

Upon further critical evaluation of the paper of Koide et al., we could appreciate several subtle differences in experimental set-up that explain most of the apparent discrepancies. For instance, most of the experiments Koide et al. presented were performed in the presence of exogenous Shh. As a result, the effect of Ptch1 on Smo activity was not taken into account and the inhibitory role of vitamin D3 (inhibiting Smo after translocation across the cell membrane) was thus overlooked. In such a model system, changing the levels of intracellular 7-DHC and, consequently, of vitamin D3 by modulating DHCR7 levels does not increase the extracellular vitamin D3 concentration and cannot mediate Smo inhibition. It would be interesting to see the effect of DHCR7 knock down on Hedgehog pathway reporter activity in the absence of Shh.

Another complicating factor in the interpretation of the effects of DHCR7 knock down on Hedgehog pathway activity is the ectopic expression of Shh the authors described after DHCR7 morpholino injection [see figures 5 and 6 in Koide et al. (Koide et al., 2006)]. As Shh is not a known target gene of the Hedgehog pathway, this upregulation is puzzling and provides another source of Shh to paralyze Ptch1 in its action as described above. In this regard, it is important to note that in our own experimental set-up, we have aimed to eliminate the contribution of Shh to be able to focus on the action of Ptch1, and once more the differences between models systems in studying Hedgehog signal transduction become apparent.

Although (or maybe because) Koide et al. circumvented Ptch1 inhibitory activity in their model system, it was elegantly shown that the reductase activity of DHCR7 is not necessary for the inhibitory action on the Hedgehog pathway. Using various DHCR7 mutant constructs, Koide et al. show that the inhibitory activity of DHCR7 is mediated via the N-terminal domain of DHCR7 (Koide et al., 2006). These results should, however, be interpreted with care, as one should realize that the deletion of a large N-terminal part of a protein might remove essential signal- and start-transfer sequences, thereby perturbing the orientation of the protein in the membrane.

Combining the data of Koide et al. with our own suggests that DHCR7 plays a dual role in Hedgehog signalling. Through its enzymatic activity, it limits vitamin D3 levels in the cell, thereby reducing vitamin D3 secretion via Ptch1 and, subsequently, Ptch1-dependent Smo inhibition. Alternatively, via a currently unknown mechanism but independently of its enzymatic activity, DHCR7 functions as a negative regulator of Shh signalling. The net effect and physiological relevance of these opposing activities of DHCR7 seems a challenging dilemma that needs to be resolved in the near future. Also, as the authors point out themselves, the duality of DHCR7 action on Hedgehog pathway activity is reflected in the different, often incomprehensible, phenotypes of Smith-Lemli-Opitz syndrome (SLOS) patients.

References

Bijlsma, M. F., Spek, C. A., Zivkovic, D., Van de Water, S., Rezaee, F. and Peppelenbosch, M. P. (2006). Repression of Smoothened by Patched-dependent (pro-)vitamin D3 secretion. *PLoS Biol.* **4**, e232

Cooper, M. K., Wassif, C. A., Krakowiak, P. A., Taipale, J., Gong, R., Kelley, R. I., Porter, F. D. and Beachy, P. A. (2003). A defective response to Hedgehog signaling in disorders of cholesterol biosynthesis. *Nat. Genet.* **33**, 508-513.

Koide, T., Hayata, T. and Cho, K. W. (2006). Negative regulation of Hedgehog signaling by the cholesterogenic enzyme 7-dehydrocholesterol reductase. *Development* **133**, 2395-2405.

Maarten F. Bijlsma*, Maikel P. Peppelenbosch and C. Arnold Spek

Center for Experimental and Molecular Medicine, Academic Medical Center, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands
*Author for correspondence (e-mail: m.f.bijlsma@amc.uva.nl)

RESPONSE

More challenges ahead of DHCR7's role in Hh signaling

Smith-Lemli-Opitz syndrome (SLOS) is caused by defects in 7-dehydrocholesterol reductase (DHCR7), a key enzyme in the final step of cholesterol biosynthesis. Cholesterol has been suggested to play roles in Hedgehog (Hh) signaling by either the direct cholesterification of Hh ligands or by regulating the function of Smoothened (Smo), a hedgehog pathway inducer. Many developmental malformations attributed to SLOS occur in tissues and organs in which Hh signaling is required for development. However, the precise role of DHCR7 deficiency in this disease remains confusing. Our recent findings (Koide et al., 2006) in *Xenopus*, and a recent report by Bijlsma et al. (Bijlsma et al., 2006), underscore the complexity of DHCR7 function in Hh signaling and the condition of SLOS.

Before we consider the implications of these findings further, we would like to address certain statements in the Bijlsma et al. Correspondence regarding the role of DHCR7 in *Xenopus*. We examined the role of DHCR7 in Hh signaling in several tissues during early *Xenopus* embryogenesis. We found that DHCR7 appears to function in an inhibitory fashion, paradoxical to the mainstream view of its role in Hh signaling. Further investigation will be necessary to determine how broadly DHCR7 functions in this way. In addition, our paper concludes that the reductase activity of DHCR7 is 'dispensable' for its inhibitory action, not 'indispensable' as stated by Bijlsma et al. in their Correspondence. Our model also suggests that DHCR7 may act in the receiving cells either at the level, or downstream, of Smo, and seemingly upstream of Gli. Lastly, because our manuscript was published before the Bijlsma et al. paper (Bijlsma et al., 2006), we were unaware of the possible effects of vitamin D3 on Smo. However, in light of this new and important observation, it is useful to examine the experimental approaches, results and conclusions of these papers to determine whether one model can take into account the findings of these two new studies.

Phenotypes of DHCR7-defective embryos

As noted by Bijlsma et al., impaired function of DHCR7 might be expected to lead to accumulation of this enzyme's substrate, 7-DHC (7-dehydrocholesterol). According to their hypothesis, a build up of 7-DHC would subsequently be converted to vitamin D3, which their new data suggest functions as a direct Smo antagonist. Thus, DHCR7 should function as a positive regulator of Hh signaling, which is consistent with the common opinion held in the field. However, current embryological phenotypes of loss of DHCR7 in both mice and *Xenopus* are not consistent with this simple view. Although DHCR7-deficient mutant mice (deficient in reductase activity due to the elimination of specific exons) display cholesterol deficiency (showing both accumulation of 7-DHC and low cholesterol), they fail to show any obvious defects consistent with a loss of Hh signaling (Fitzky et al., 2001; Waage-Baudet et al., 2003; Wassif et al., 2001; Yu et al., 2004). Furthermore, we have found that the overexpression of DHCR7 inhibits Hh signaling in *Xenopus* embryological assays and, consistent with this observation, that the knockdown of DHCR7 by DHCR7 morpholino injection promotes Hh signaling. These findings together imply that the function of DHCR7 in Hh signaling is complex and that DHCR7 has other roles in addition to its previously anticipated role in cholesterol biosynthesis.

Reductase activity of DHCR7 in early frog development

Our analyses using DHCR7 *Xenopus* mutants defective in reductase activity, as well as our use of a pharmacological inhibitor of the reductase, suggest that DHCR7's negative effects on Hh signaling are independent of its enzymatic activity. Whether this reductase-independent inhibitory effect is observed only during early *Xenopus* embryogenesis, or whether it occurs at later stages of development requires further investigation. In this regard, it would be important to find out when de novo cholesterol biosynthesis occurs during *Xenopus* embryogenesis. Tint et al. (Tint et

al., 2006) recently reported that most of the cholesterol accumulated in early mouse embryos is maternal in origin, and that endogenous cholesterol synthesis in embryos rapidly increases after E10-E11 in the brain, and E12-E14 in the liver and lung. A similar scenario can be imagined for the early frog embryo, as much of the material required for early embryogenesis is packed into the egg maternally during oogenesis. Hence, we can imagine a situation in which the reductase activity of DHCR7 is dispensable for early embryos, and thus the loss of DHCR7 may not lead to an accumulation of 7-DHC or vitamin D3 to inhibit Smo during early development.

Vitamin D3 and 7-DHC

It seems quite reasonable to extrapolate the model of Bijlsma et al. that mouse Ptch1 acts in the secretion of vitamin D3, and that this is the general mechanism by which Patched negatively regulates Smo activity. Bijlsma et al.'s treatment of zebrafish embryos with vitamin D3 (Bijlsma et al., 2006) is consistent with their model, giving rise to Smo loss-of-function phenotypes. However, the effects of 7-DHC and the role of DHCR7 on this vitamin D3 effect in developing zebrafish embryos was not studied. Additionally, the exogenously applied concentrations of vitamin D3 appear to be quite high, and therefore the physiological relevance of these experiments is difficult to assess at present. Finally, as noted by Bijlsma et al., vitamin D3 production from 7-DHC requires exposure to UV light. It is of interest to note that *Xenopus* and zebrafish embryos develop perfectly normally in the dark (in the complete absence of UV light). Hence, either vitamin D3 cannot be photoconverted from 7-DHC and thus this process is unlikely to play a major role in affecting Hh signaling during early embryogenesis, or vitamin D3 may be maternally accumulated in early *Xenopus* and zebrafish embryos. This is another important question to examine in the future.

Isoforms of DHCR7

Recently, three isoforms of DHCR7 have been isolated in *Xenopus*, and splice variants

of DHCR7 in rats and mice have also been identified (Tadjuidje and Hollemann, 2006; Lee et al., 2002). Interestingly, the *Xenopus* isoforms do not display identical phenotypes when assayed by overexpression (Tadjuidje and Hollemann, 2006), raising the possibility that different forms of DHCR7 may display different activities. In light of the apparent dual function of DHCR7, it is tempting to speculate that different forms of DHCR7 are expressed in different tissues, and that these display different levels of reductase and Hh antagonistic activities. The difference between our results in *Xenopus* embryos and Bijilsam et al.'s in cell culture could be partly explained by the differential expression of different DHCR7 isoforms that possess different activities.

Future studies

In light of these new findings on the role of Ptch1 on Smo via vitamin D3, our future work should include an examination of the role of vitamin D3 during *Xenopus* embryogenesis, and whether the manipulation of DHCR7 expression influences the availability of vitamin D3 to regulate Hh signaling. Additionally, it would be useful to examine Gli-reporter gene activity in the DHCR7 morpholino-injected embryos in the absence of Shh

signaling, as our assay was always done in the presence of exogenous or endogenous Shh signaling. Lastly, using various DHCR7 deletion and point mutations, we found that the inhibitory role of DHCR7 can be uncoupled from the reductase enzymatic activity. While the result is supportive of the notion that the inhibitory activity is reductase independent and mediated via the N-terminal of DHCR7, it is not conclusive because tampering with the structure of any protein could result in a change in protein activities for various reasons. Therefore, further mutational analyses of DHCR7, together with structural studies, should be performed to better define the role of DHCR7.

References

Bijlsma, M. F., Spek, C. A., Zivkovic, D., Van de Water, S., Rezaee, F. and Peppelenbosch, M. P. (2006). Repression of Smoothened by Patched-dependent (pro-)vitamin D3 secretion. *PLoS Biol.* **4**, e232.

Fitzky, B. U., Moebius, F. F., Asaoka, H., Waage-Baudet, H., Xu, L., Xu, G., Maeda, N., Kluckman, K., Hiller, S., Yu, H. et al. (2001). 7-Dehydrocholesterol-dependent proteolysis of HMG-CoA reductase suppresses sterol biosynthesis in a mouse model of Smith-Lemli-Opitz/RSH syndrome. *J. Clin. Invest.* **108**, 905-915.

Koide, T., Hayata, T. and Cho, K. W. (2006). Negative regulation of Hedgehog signaling by the cholesterogenic enzyme 7-dehydrocholesterol reductase. *Development* **133**, 2395-2405.

Lee, J. N., Bae, S. H. and Paik, Y. K. (2002). Structure and alternative splicing of the rat 7-dehydrocholesterol reductase gene. *Biochim. Biophys. Acta* **1576**, 148-156.

Tadjuidje, E. and Hollemann, T. (2006). Cholesterol homeostasis in development: the role of Xenopus 7-dehydrocholesterol reductase (Xdhcr7) in neural development. *Dev. Dyn.* **235**, 2095-2110.

Tint, G. S., Yu, H., Shang, Q., Xu, G. and Patel, S. B. (2006). The use of the Dhcr7 knockout mouse to accurately determine the origin of fetal sterols. *J. Lipid Res.* **47**, 1535-1541.

Waage-Baudet, H., Lauder, J. M., Dehart, D. B., Kluckman, K., Hiller, S., Tint, G. S. and Sulik, K. K. (2003). Abnormal serotonergic development in a mouse model for the Smith-Lemli-Opitz syndrome: implications for autism. *Int. J. Dev. Neurosci.* **21**, 451-459.

Wassif, C. A., Zhu, P., Kratz, L., Krakowiak, P. A., Battaile, K. P., Weight, F. F., Grinberg, A., Steiner, R. D., Nwokoro, N. A., Kelley, R. I. et al. (2001). Biochemical, phenotypic and neurophysiological characterization of a genetic mouse model of RSH/Smith–Lemli–Opitz syndrome. *Hum. Mol. Genet.* **10**, 555-564.

Yu, H., Wessels, A., Chen, J., Phelps, A. L., Oatis, J., Tint, G. S. and Patel, S. B. (2004). Late gestational lung hypoplasia in a mouse model of the Smith-Lemli-Opitz syndrome. *BMC Dev. Biol.* **4**, 1.

Tetsuya Koide, Tadayoshi Hayata and Ken W. Y. Cho*

Department of Developmental and Cell Biology, University of California, Irvine, CA 92697-2300, USA
*Author for correspondence (e-mail: kwcho@uci.edu)