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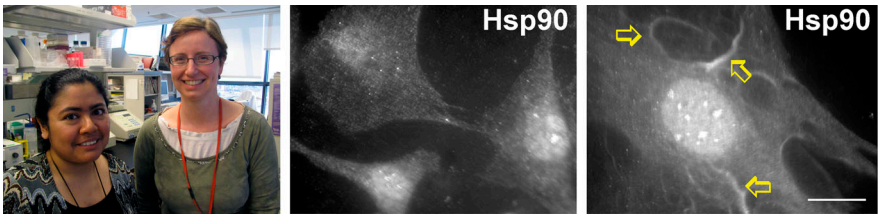
Directing a chaperone

Study suggests that cranial mesenchyme cells must limit Hsp90 secretion during development.

Focal Point



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Anjali Sarkar (left) and Irene Zohn (right) reveal that the ubiquitin ligase Hectd1 restricts the migration of cranial mesenchyme cells by inhibiting secretion of the protein chaperone Hsp90. Hsp90 is found in the nucleus and cytoplasm of wild-type cells (center image) but is found on the surface of cranial mesenchyme cells lacking functional Hectd1 (far right, arrows). Excess extracellular Hsp90 enhances the motility of Hectd1-deficient cells, potentially explaining the abnormal organization of the cranial mesenchyme and neural tube closure defects seen in *Hectd1* mutant mouse embryos.

Early in development, the central nervous system originates from the neural plate, which folds on itself to form the neural tube. In the cranial region, this complex morphogenesis also requires forces generated in the epithelium and mesenchyme that surround the neural tissue. The edges of the neural plate, for example, are initially pushed upwards by the underlying cranial mesenchyme (CM), which expands by altering the extracellular matrix to displace CM cells further away from each other (1). Sarkar and Zohn report that this tissue rearrangement depends on a ubiquitin ligase that restricts secretion of the chaperone protein Hsp90 (2).

Errors in neural tube closure cause some of the most common birth defects in humans (3). In a mutagenesis screen she performed as a postdoc with Lee Niswander, Irene Zohn found that mouse embryos lacking the ubiquitin ligase Hectd1 failed to close their cranial neural tubes properly, possibly because their CM cells remain close together instead of moving apart (4). "Once we found the *Hectd1* gene, we wanted to understand the molecular basis of the phenotype," says Zohn, now a group leader at the Children's National Medical Center in Washington, DC. "Since Hectd1 is a ubiquitin ligase, we started looking for its substrate."

Zohn and postdoc Anjali Sarkar looked for Hectd1-interacting proteins by co-immunoprecipitation and a yeast two-hybrid screen (2). Both approaches identified the molecular chaperone Hsp90 as a binding partner, and therefore potential substrate, of the ubiquitin ligase. Indeed, Sarkar and Zohn found that Hectd1 polyubiquitinated Hsp90 in vivo and that this modification was reduced in CM cells from mice lacking functional Hectd1.

> **"The excess extracellular Hsp90 may alter the interaction of cranial mesenchyme cells with the extracellular matrix."**

The chain of ubiquitin molecules added to Hsp90 by Hectd1 were linked via the lysine residues at position 63 of each ubiquitin monomer, a connection usually associated with changes in protein localization or function rather than with protein degradation. The researchers looked at Hsp90's localization and found that the chaperone was in the nucleus and cytoplasm in wild-type CM cells, whereas it was found at the external face of the plasma membrane in the absence of Hectd1.

Hsp90 mostly functions intracellularly, but it can also be secreted from cells inside small vesicles called exosomes (5). Sarkar and Zohn found that the chaperone's export was reduced in cells expressing wild-type, but not catalytically inactive, Hectd1. Once outside the cell, Hsp90 can promote cell migration, probably by modifying the conformation of cell-surface receptors and extracellular matrix proteins (6). Sarkar and Zohn therefore wondered whether the excess Hsp90 secreted by Hectd1-deficient cells might alter their motility. "When we explanted cranial mesenchyme, more cells left *Hectd1* mutant explants than wild-type explants, and the mutant cells migrated farther than wild-type cells," explains Zohn. This enhanced motility was due to the increase in Hsp90 secretion: blocking extracellular Hsp90 with an inhibitory antibody reduced the migration of Hectd1-deficient cells, whereas the motility of wild-type cells was boosted by the addition of recombinant Hsp90 to the medium, replicating the *Hectd1* mutant phenotype.

"There may be other pathways involved in the failure of neural tube closure, because we're finding that Hectd1 has a lot of substrates," says Zohn. "But it seems that excess Hsp90 secretion can model a lot of the abnormal behavior that we see in mutant CM cells." It remains to be seen how ubiquitination inhibits Hsp90's export from the cell—Zohn thinks that the modification may traffic the chaperone away from the endosomes and lysosomes where exosomes are formed.

The increased motility of ligase-deficient cells may help explain their abnormal organization and the lack of CM expansion in *Hectd1* mutant embryos. "The excess extracellular Hsp90 may alter the interaction of cranial mesenchyme cells with the extracellular matrix, resulting in a failure of mutant CM cells to expand," says Zohn. "So now we're trying to figure out what Hsp90's extracellular clients are."

1. Copp, A.J., et al. 2003. *Nat. Rev. Genet.* 4:784–793.
2. Sarkar, A.A., and I.E. Zohn. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201105101.
3. Zohn, I.E., and A.A. Sarkar. 2008. *Curr. Top. Dev. Biol.* 84:1–35.
4. Zohn, I.E., et al. 2007. *Dev. Biol.* 306:208–221.
5. Hegmans, J.P., et al. 2004. *Am. J. Pathol.* 164:1807–1815.
6. Tsutsumi, S., and L. Neckers. 2007. *Cancer Sci.* 98:1536–1539.