

Trichoplein keeps primary cilia silent

Study identifies a centriolar protein that activates Aurora A to suppress ciliogenesis in proliferating cells.

Most quiescent vertebrate cells sport a primary cilium, a microtubule-based membrane protrusion that receives extracellular signals and transmits them to the rest of the cell. Ciliary microtubules are nucleated from the basal body, a structure that, in its alternative guise as a centriole, also forms the basis of centrosomes. Quiescent cells must therefore disassemble their primary cilia when they reenter the cell cycle, so that the basal body/centriole can fulfill its function in organizing the mitotic spindle. Inoko et al. reveal how proliferating cells ensure they don't regrow a primary cilium until they return to a quiescent state (1).

Masaki Inagaki and colleagues at the Aichi Cancer Center Research Institute in Nagoya, Japan, were interested in the function of trichoplein, a protein that localizes to both intercellular adhesions (2) and centrosomes (3). "We noticed that, although trichoplein localizes to centrioles in proliferating cells, it disappears from the basal body in quiescent cells," says Inagaki, referring to experiments performed in the RPE1 epithelial cell line (1).

The researchers, led by Akihito Inoko and Makoto Matsuyama, therefore wondered whether trichoplein might prevent the centrioles of proliferating cells from nucleating primary cilia. "Knocking down trichoplein induced primary cilia formation in proliferating RPE1 cells," says Inagaki. Overexpressing trichoplein, on the other hand, inhibited cilia assembly in quiescent cells.

When cells reenter the cell cycle, cilia resorption is driven by the mitotic kinase Aurora A and two activating proteins called HEF1 and Pitchfork (4, 5). Aurora A phosphorylates the enzyme HDAC-6, stimulating the deacetylation and destabilization of ciliary microtubules. Inoko et al. therefore investigated whether Aurora A worked with trichoplein to suppress cilia reassembly in proliferating cells.

FOCAL POINT



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(Left to right) Masaki Inagaki, Akihito Inoko, Makoto Matsuyama, and colleagues (not pictured) reveal how proliferating cells suppress the formation of primary cilia. The centriolar protein trichoplein inhibits cilia assembly by activating the Aurora A kinase. When cells exit the cell cycle, however, trichoplein is lost from the centriole that becomes the ciliary basal body, allowing ciliogenesis to proceed in quiescent cells. Unlike control cells (second from right), proliferating RPE1 cells lacking trichoplein (far right) aberrantly assemble primary cilia (stained for acetylated tubulin, green). This results in cell cycle arrest, suggesting that the vertebrate cell cycle can be regulated by cilia assembly and resorption.

Trichoplein bound and activated Aurora A in vitro, and the two proteins colocalized at the centrioles of cycling cells. Aurora A activity was reduced at the centrioles of cells lacking trichoplein, and depleting the kinase by RNAi induced ciliogenesis in proliferating cells. In addition, a short fragment of trichoplein that could localize to centrioles and activate Aurora A was sufficient to block cilia formation in quiescent RPE1 cells.

Trichoplein therefore appears to inhibit ciliogenesis by activating Aurora A, which, similar to its role in cilia resorption, may activate HDAC-6 and destabilize microtubules. But what happens to proliferating cells when cilia reassembly isn't inhibited? Inoko et al. found that RPE1 cells lacking trichoplein or Aurora A arrested in G0/G1. "But this arrest was reversed if primary cilium formation was blocked by simultaneously

depleting [the key ciliary protein] IFT20," says Inagaki, suggesting that aberrant ciliogenesis prevents cell cycle progression.

"Only vertebrates possess primary cilia—yeast, *C. elegans*, and *Drosophila* don't have them," explains Inagaki. "So, although these model organisms have contributed to our understanding of the cell cycle, we may have to consider the impact of primary cilia to fully understand how the mammalian cell cycle is regulated." Trichoplein or Aurora A knockdown failed to induce ciliogenesis or cell cycle arrest in HeLa cells, which, like many cancerous cell types, have a reduced tendency to form primary cilia. "We're now investigating whether Aurora A knockdown has different effects in various normal and cancer cell lines," says Inagaki. Because Aurora A is required for mitotic spindle assembly, inhibiting the kinase could cause fatal mitotic errors in tumor cells, whereas healthy cells would merely assemble cilia and exit the cell cycle, potentially making Aurora A an attractive target for anti-cancer therapies.

Inagaki and colleagues are also interested in how trichoplein is removed from the basal bodies of quiescent cells in order to permit cilia assembly. "Trichoplein may be degraded by a centriolar E3 ubiquitin ligase," Inagaki says. "We have some candidates that we're analyzing now."

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