

Cancer as a Ciliopathy: The Primary Cilium as a New Therapeutic Target

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Introduction

The primary cilium versus motile cilia

Research on primary cilium is taking an increasing interest, both for scientists engaged in developmental biology, and for those dedicated to cancer research. Decades ago, the primary cilium was considered to be a vestigial organ of embryonic development. Its structure is different from that of motile cilia. The latter present a structure of 9 pairs of microtubules plus 2 central microtubules; primary cilia only consist of 9 pairs of microtubules. Primary cilia modulate several functions

related to cell differentiation and signaling. The study of primary cilia has nowadays become of impact, both in terms of developmental biology, within ciliopathies [1], and in cancer [2]. In this sense the cilium is understood as an anchor cellular structure for molecules that are processed into it, being transported up and down the cilium by kinesin and dynein proteins.

Sonic hedgehog: a molecular pathway linked to the primary cilium

In this respect, the sonic hedgehog (Hh) pathway has been documented to be related to primary cilia [2]. Before taking into account the function of primary cilia in cancer development, we will summarize the regulation of the Hh pathway (Table 1).

	Primary cilium is present: correct ciliogenesis				Absence of primary cilium: incorrect ciliogenesis			
	Will GliA be produced in the cilium?	Gli target genes	Cell growth	Therapy	Will GliA be produced in the cilium?	Gli target genes	Cell growth	Therapy
ligand overexpression (SHH, DHH, IHH)	YES	ON	increased	robotnikinin	NO	OFF	Not increased	Not required
SMO activating mutations	YES	ON	increased	cyclopamine vismodegib	NO	OFF	Not increased	Not required
PTCH inactivating mutations	YES	ON	increased	cyclopamine vismodegib	NO	OFF	Not increased	Not required
Gli1 gene amplification	NOT, but Gli1 proteins overexpressed from Gli1 gene amplification will be the GliA forms	ON	increased	GANT	NO, but Gli1 proteins overexpressed from Gli1 gene amplification will be	ON	increased	GANT

Table 1: Hh pathway regulation in two different conditions (with and without primary cilium): implications for specific treatments.

The most important molecules to be considered within the Hh pathway are the Hh ligands (SHH, DHH and IHH; for Sonic, Desert and Indian Hedgehog, respectively), PTCH ligand receptor (a transmembrane receptor that acts as a tumor suppressor gene), the SMO receptor (a G protein-coupled receptor that acts as an oncogene), and the Gli proteins (1, 2, and 3), of which 1 and 2 are considered to act as GliA (Gli activator proteins), while Gli3 is the main Gli repressor (GliR).

Trying to illustrate the regulatory mechanisms of the Hh pathway, we may say the following: PTCH inhibits SMO when PTCH is free of its ligand (SHH, DHH or IHH). Ligand binding to PTCH releases the inhibition of PTCH on SMO, leaving SMO in an active form to induce Gli activation, and thus make active Gli induce the expression of its

downstream target genes, such as cyclins, MYC, PTCH, etc. Hh in a hyperactive status will induce cell growth and cancer development.

SHH, SMO and GLI behave as oncogenic products, as they lead to cell cycle activation. On the contrary PTCH acts as a tumor suppressor gene, as it inhibits SMO. Inactivating mutations in PTCH, or activating mutations in SMO contribute to Hh activation and cell growth promotion. GLI1 gene amplification and ligand overexpression are the other two typical alterations that would enhance Hh activity.

But this description of the regulation of the Hh pathway will not be sufficient to understand the whole picture, unless we count with the role of the primary cilium to better explain Hh regulation [2]. When SMO gets active, after ligand binding to PTCH, SMO moves to the cilium, where it can execute its function: production of Gli active

forms (GliA), mainly Gli2. If the primary cilium is not present, SMO can do nothing to produce GliA. The cilium is required for SMO to form GliA and have Hh activated to promote cell growth. So, we might say that an active SMO plus the presence of a primary cilium will produce GliA and Hh activation, while the contrary is usually true as well: an active SMO without a primary cilium will not produce GliA, so Hh will get inactive. There is one exception to this second rule: the presence of GLI1 gene amplification. In this context, a major proportion of GliA overexpressed from the GLI1 amplified gene (and not produced by SMO activity in the cilium, as there is no cilium) versus that of GliR existing in the cytoplasm will produce Hh activation.

In summary, we might almost take as a rule the next statements (Table 1): first, the presence of primary cilium associates to cancer formation if any or several of the next alterations are present: Hh ligand overexpression, PTCH inactivating mutations, or SMO activating mutations; second, the absence of cilium lead us to think that no cancer is going to be produced by activation of the Hh pathway by any or several of the alterations already referred above; and third, the exception to this rule would be the absence of primary cilium together with Gli1 gene amplification, a common molecular alteration in gliomas [3]: in such a condition, Gli overexpression of GliA forms would be produced without requiring the presence of the primary cilium; therefore we would have a Hh-linked cancer in cells that do not have a primary cilium.

All these conditions would require different specific therapeutic approaches [2]. It is well known that cyclopamine and vismodegib are active SMO inhibitors, while robotnikinin is an inhibitor of Hh ligands, and GANTs are inhibitors of GliA forms. A tumor presenting primary cilia and Hh activation by Hh ligand overexpression, PTCH mutations, or SMO mutations, should be specifically treated according to the lesion presented. But it is remarkably important to underline that this would not be true if primary cilia were not present; in such a case no treatment targeting the Hh pathway would be required against Hh ligand overexpression, PTCH mutations, or SMO mutations, as none of those three alterations or any combinations of them would be able to increase cell growth in cells without primary cilia required for GliA production. Only the exceptional case of cells without primary cilia and with Gli1 gene amplification should be treated with GANTs and with no other of the described specific treatments for each specific lesion of the Hh pathway.

Is the primary cilium a cancer repressor or a cancer promoter?

The association between cancer and presence of primary cilium is not as straightforward as it seems at first sight. There is a line of scientific thinking that defends that cilia might repress tumor formation. One example is the VHL tumor suppressor gene, that seems to maintain cilia [4,5]. Therefore VHL mutations, producing Von Hippel Lindau disease, an inherited condition whose patients suffer from renal cell carcinoma, would reduce cilia; truly, cells of the renal premalignant cysts of VHL disease lack cilia.

We can see a second example in the articles published by Mooser et al., [6,7], where they demonstrate by electron microscopy that glioblastoma cells and tumors do not present primary cilia, and they even propose that primary ciliogenesis is disrupted at an early stage in glioblastoma, maybe involving protein Cap123.

Thirdly, Sarkisian et al., [8] give some clues on the molecular aspects of ciliogenesis by demonstrating that protein CCRK, a positive regulator of the cell cycle, is abnormally high in cells that lack primary cilia (U251 glioblastoma cell line), and that knockdown of CCRK in U251 cells induced ciliogenesis and inhibited U251 proliferation, which seems to demonstrate that cilia might repress tumor formation.

On the other side, some studies make us think that the presence of cilia is required for cancer development. Sarkisian et al., [8] saw primary cilia in glioblastoma cells and tumors, but only in those cells which are more proliferative, more invasive and resistant to chemotherapy. Secondly, knowing that Kif3A is a protein required for ciliogenesis, it has been demonstrated that Kif3A knockdown inhibits cilia and inhibits medulloblastoma and basal cell carcinoma growth [9-11]. Thirdly, Emoto et al., [12] could determine the presence of cilia in 25% of pancreatic ductal carcinoma tumor samples, just the tumors associated to worse prognosis.

These three last papers show that at least a part of the cells that constitute the tumor mass might have cilia. Even, those ciliated cells seem to be prognostically worse. More studies should be done in this respect, in order to separate populations of cancer cells with and without cilia, and see the differences between them. The cilia-containing cells might be associated to the cancer stem cell (CSC) phenotype, as CSC show an increase of proliferation, migration and resistance to chemotherapy, as the ciliated tumor cells of some publications seem to show [8,12].

Whether ciliated cancer cells associate to CSC or not, is something so far undetermined, and requires further research at the morphological and molecular levels of the primary cilium, to help doctors establish a better clinical prognosis and treatment for the patients. A second interesting question regarding cilia research might concern the various different molecular pathways (not just Hh) that might depend on cilia to execute their functions [13,14]. Finally, an ample study on tumors derived from the different blastodermic layers, including carcinomas, sarcomas, leukemia-lymphoma, neuroectodermal tumors, and those derived from the neural crest (both from ectoderm and mesoderm), would be very beneficial to evaluate the role of the primary cilium along many different tumors of different origin and with different mutations; a question of high importance to test the hypothesis of cancer as a ciliopathy.

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