

Research Highlights

aPKC ι/λ : a potential target for the therapy of Hh-dependent and Smo-inhibitor-resistant advanced BCC

Xiangdong Lv, Lin Fu, and Yun Zhao*

State Key Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

*Correspondence address. Tel: +86-21-54921618; E-mail: yunzhao@sibcb.ac.cn

Basal cell carcinoma (BCC), a skin cancer, is the most common malignancy in white people [1, 2]. Although mortality is low as BCC rarely metastasizes, people suffering from BCC are at high risk of developing other malignancies [3]. Given its low metastatic potential, treatment of BCC focuses on local control, including surgical and non-surgical measures [3, 4]. In the best hands, the 5-year cure rates of excision, curettage and cautery, and cryosurgery are 95% or higher [3].

However, considering multiple postsurgical recurrences or incurable with surgery without significant deformity or loss of function, locally advanced BCC is inoperable, and so is metastatic BCC [5]. Therefore, looking for inhibitors of specific targets for therapy of BCC based on its molecular pathogenesis is of great importance. Although Vismodegib, approved as the first medicine for adults with advanced BCC (locally advanced BCC and metastatic BCC) by US Food and Drug Administration (FDA) in 2012, appears effective in the treatment of BCC, treatment-driven evolution has resulted in the outgrowth of tumor cell variants resistant to the drug [5]. New targets for BCC treatment are necessary [6–9]. Recently, Atwood *et al.* [9] reported in *Nature* that aPKC ι/λ inhibition would be a viable, tumor-selective alternative to Smo inhibitors, Vismodegib, to treat BCC.

Thinking about the severe consequence and the high incidence of BCC, scientists have tried their best to investigate the pathogenesis, and enormous progress has been made in its genetic and molecular mechanism. It has been showed that almost all of the BCCs contain genetic mutations in Hedgehog (Hh) signaling pathway, resulting in aberrant pathway activity and uncontrolled proliferation of basal cells [8]. Molecular and genetic studies further clarified that the growth of BCCs requires the high activity of Hh signaling pathway through the transcription factor, glioma-associated oncogene homolog (GLI) [7]. Based on this, people have tried to translate these insights into improving BCC treatment by targeting Hh signaling pathway. Some clinical trials of inhibitors of Hh signaling pathway have indicated that

disruption of Hh signal in tumors can result in therapeutic benefit. Among these inhibitors, Vismodegib, an antagonist of Smo, has been shown to associate with tumor responses in patients with advanced BCC [8] and was approved as the first drug for BCC treatment on January 30, 2012 by US FDA. However, advanced tumors can evolve resistance through pathway-dependent genetic mechanisms or through compensatory adaptation [6]. Some tumor variants can bypass Smo inhibitors and still activate Hh signal, resulting in the secondary (acquired) resistance to the drug [5]. Because the growth of tumor variants with resistance to Vismodegib is also Hh activity-dependent, the most downstream transcription factor GLI or direct regulator(s) of GLI could be potential targets.

In order to identify new potential target(s) in Hh pathway for BCC, Atwood *et al.* used miss in metastasis (MIM), which potentiates GLI-dependent activation downstream of Smo [10], as bait in a biased proteomics screen to identify factors involved in Hh signaling and ciliogenesis. Two of the hits were polarity proteins which were not previously linked to the Hh pathway: aPKC- ι/λ , a serine/threonine kinase, and PARD3, a PKC- ι/λ substrate [9]. The subsequent experiments mainly employed BCC cells as a niche to mimic BCC physiological status. First of all, they performed co-immunoprecipitation assay followed by western blot with anti-MIM antibody or anti-aPKC- ι/λ antibody to confirm the interaction between MIM and aPKC- ι/λ in BCC cells. Then, they found that the knocking down of MIM or aPKC- ι/λ with short hairpin RNA leads to the down-regulation of messenger RNA (mRNA) level of *Gli1*, which is the direct target gene of Hh signaling pathway. In addition, the inhibition of aPKC- ι/λ kinase activity with myristoylated aPKC peptide inhibitor (PSI) [11] resulted in the decrease of *Gli1* mRNA level in a dose-dependent manner, and the growth of the BCC cell was also inhibited at the same condition according to the MTT assay, which has been used widely to measure the cell growth rate. Then, they showed that PSI specifically inhibited aPKC based on the finding

that the loss of aPKC- ι/λ in BCC cells in combination with PSI treatment possessed no additional activity to reduce *Gli1* mRNA level [9]. These results indicated that aPKC- ι/λ , as a centrosome-associated protein, regulates Hh signaling [9].

Because aPKC- ι/λ is essential for the maximal Hh signaling, it is of great interest to test whether the expression of this gene is upregulated in BCCs. The mRNA and protein level of aPKC- ι/λ were detected by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) and immunostaining, respectively, and the results revealed that not only the total but also activated aPKC- ι/λ is upregulated. In the subsequent experiments, the possibility that aPKC- ι/λ is the direct target of Hh signal is tested. RT-qPCR analysis showed that the mRNA level of aPKC- ι/λ correlates with Hh signal, which is similar to that of *Gli1*. Chromatin immunoprecipitation assay with anti-Flag antibody to pull down over-expressed Flag-Gli1 in BCC cell indicated that *Prkci* (gene name of aPKC- ι) is a direct target gene of Gli1. Taken together, it was suggested that aPKC- ι/λ is part of Hh-mediated positive feedback loop leading to its expression upregulation in human and mouse BCCs [9]. In order to further figure out the regulation mechanism of aPKC- ι/λ on the growth of BCCs, they performed RNA-seq of SANT-1 (inhibitor of Smo) or PSI-treated samples to test whether aPKC- ι/λ -dependent genes in BCCs differed from those regulated by Smo. Briefly, by comparing the genes whose transcription level were changed 2 fold or more, they found that ~90.82% of the aPKC- ι/λ -dependent genes are also regulated by Smo and that most of these genes are involved in cell cycle regulation, protein transport, protein localization, and cell division. These data have substantial overlap with previously published Hh-dependent data from mouse medulloblastomas/granular neural precursors or developing mouse limb bud. These results indicated that aPKC- ι/λ and Smo regulate a common set of Hh target genes in BCCs. The mechanistic study revealed that aPKC- ι/λ -catalyzed Gli1S243T304 phosphorylation activates *Gli1* by promoting Gli1 binding to its target DNA, while the stability or localization is not affected [9].

What is the biological function of aPKC- ι/λ in the growth control of BCC cell? Is it suitable for BCC treatment? The authors further tested this possibility. The allografted BCC tumors, which can accurately describe human BCCs and serve as a model to validate Hh inhibitors in clinical, were chosen for the subsequent investigation. *Gli1* mRNA level was decreased, when treated with PSI, and the tumor size was suppressed at the same situation without any apparent acquired resistance. This effect was comparable with that of Smo antagonist itraconazole or GLI2 inhibitor arsenic trioxide. Because some tumor variants are resistant to the Smo inhibitor, Vismodegib, they wondered whether PSI could do something with the Smo-resistant tumors. They generated multiple independent BCC cell lines that were resistant to SANT-1 at high levels and found that aPKC- ι/λ expression was elevated

in these cell lines. The resistant cell lines were verified by high concentration cyclophosphamide treatment, and little effect on cell proliferation was found. However, the cell proliferation was inhibited by PSI very dramatically. The *in vivo* comparison of local Smo-inhibitor-sensitive and Smo-inhibitor-resistant invasive human BCCs demonstrated the increase of activated aPKC- ι/λ levels as a mode of resistance. Taken together, these data indicated that Smo-inhibitor-resistant BCCs' growth depends on aPKC- ι/λ by activating Hh pathway, and the suppression of aPKC- ι/λ activity is sufficient to prevent both Smo-inhibitor-sensitive and resistant BCC progression.

Hence, combining the excellent molecular mechanism and cell-based and mouse BCC allografts-based *in vivo* functional study, Atwood *et al.* successfully demonstrated that aPKC ι/λ phosphorylates and activates Gli1 and therefore potentiates Hh signaling, and that targeting aPKC ι/λ suppresses the Hh signaling and the growth of resistant BCC cell lines. These results suggested that aPKC ι/λ is critical for Hh-dependent BCC progression and implicated aPKC ι/λ as a new, tumor-selective therapeutic target for the treatment of Smo-inhibitor-resistant BCCs. Given that the over-activation of Hh signal pathway is the most basic molecular pathogenesis, and Gli transcription factors ultimately transduce signal from the Hh ligand, targeting Gli directly or their regulators will be a good choice for the next level of therapy [6].

References

- 1 Miller SJ. Etiology and pathogenesis of basal cell carcinoma. *Clin Dermatol* 1995, 13: 527–536.
- 2 Tang JY, Mackay-Wiggan JM, Aszterbaum M, Yauch RL, Lindgren J, Chang K and Coppola C, *et al.* Inhibiting the hedgehog pathway in patients with the basal-cell nevus syndrome. *N Engl J Med* 2012, 366: 2180–2188.
- 3 Wong CS, Strange RC and Lear JT. Basal cell carcinoma. *BMJ* 2003, 327: 794–798.
- 4 Rubin AI, Chen EH and Ratner D. Basal-cell carcinoma. *N Engl J Med* 2005, 353: 2262–2269.
- 5 Chang AL and Oro AE. Initial assessment of tumor regrowth after vismodegib in advanced basal cell carcinoma. *Arch Dermatol* 2012, 148: 1324–1325.
- 6 Atwood SX, Chang AL and Oro AE. Hedgehog pathway inhibition and the race against tumor evolution. *J Cell Biol* 2012, 199: 193–197.
- 7 Ng JM and Curran T. The Hedgehog's tale: developing strategies for targeting cancer. *Nat Rev Cancer* 2011, 11: 493–501.
- 8 Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD and Solomon JA, *et al.* Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *N Engl J Med* 2012, 366: 2171–2179.
- 9 Atwood SX, Li M, Lee A, Tang JY and Oro AE. GLI activation by atypical protein kinase C ι/λ regulates the growth of basal cell carcinomas. *Nature* 2013, 494: 484–488.
- 10 Callahan CA, Ofstad T, Horng L, Wang JK, Zhen HH, Coulombe PA and Oro AE. MIM/BEG4, a Sonic hedgehog-responsive gene that potentiates Gli-dependent transcription. *Genes Dev* 2004, 18: 2724–2729.
- 11 Standaert ML, Bandyopadhyay G, Perez L, Price D, Galloway L, Poklepovic A and Sajan MP, *et al.* Insulin activates protein kinases C- ζ and C- λ by an autophosphorylation-dependent mechanism and stimulates their translocation to GLUT4 vesicles and other membrane fractions in rat adipocytes. *J Biol Chem* 1999, 274: 25308–25316.