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Scaffold protein Scribble potently regulates the Sonic Hedgehog (SHH) pathway by modulating the phosphorylation and stability of the transcription factor Gli2

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In cerebellar development, the appropriate activation of SHH pathway promotes granule neurons development by controlling their progenitors' (GNP) proliferation, while its aberrant activation promotes SHH-Medulloblastoma (MB), one of the most common brain tumors in young children and adults. The genetic lesions producing SHH-MB are well known. However, novel therapeutic targets that regulate the SHH pathway are needed, as the outcomes of current therapies are not ideal. Scribble (Scrib), a member of a conserved scaffold complex, Scribble complex, is known to maintain cell polarity. Emerging studies have uncovered Scrib's roles in some important signaling pathways (e.g., MAPK/ERK), but its role in the SHH pathway is unknown. We have previously shown that 1) Scrib is highly expressed in proliferating GNPs in WT murine cerebella and purified SHH-MB tumor cells, revealing a relationship between Scrib expression and the SHH pathway activation; 2) *SCRIB* knockdown (KD) in human SHH-MB cell lines, DAOY and UW228, dramatically reduced their proliferation *in vitro*; 3) In NIH-3T3 cells (a biological tool typically used to study SHH signaling), Scrib affected the nuclear expression of Gli2, an essential transcription activator of SHH targets (like *Gli1*). To affirm the findings in NIH-3T3 cells, we stably expressed Scrib in *Scrib*KO cells at levels comparable to WT. Re-expression of Scrib restored both protein and mRNA level of Gli1, and rescued the expression and activation of nuclear Gli2. In accord with the observations in NIH-3T3 cells, the level of nuclear GLI2 was decreased with *SCRIB* KD in DAOY and UW228 cells. Furthermore, Gli2 stability requires Scrib as indicated by protein turnover studies. We hypothesized that Scrib regulates Gli2's stability and nuclear translocation by affecting its phosphorylation as a direct interaction between Scrib and Gli2 was not seen. Phospho-proteomic analysis was performed in NIH-3T3 WT and *Scrib*KO cells after treatment with SAG, a SHH pathway agonist, at different intervals (0, 1, 4, 14h). We discovered phosphorylation of 5 residues within Gli2 (S230, S232, S238, S1087 and T390) were strikingly altered by Scrib loss. To determine if phosphorylation of these sites were required for the SHH pathway activation, serine/threonine was substituted with alanine, a non-phosphorylated mutation, in a 3xHA-Gli2 construct. Constructs with WT Gli2 or Gli2 mutants were then introduced into NIH-3T3 cells with suppressed endogenous *Gli2* expression and the activation of SHH pathway was assessed. The phosphorylation of S230 and S232 of Gli2 appeared vital to SHH pathway activation. We then used an APEX tagged Scrib proximity-labeling strategy to determine Scrib's interactors in this scenario. Strikingly, Gli1, Gli2 and CK1 (a kinase reported to phosphorylate S232) appeared in the neighborhood of APEX-Scrib during SHH pathway activation. Importantly, a kinase activity prediction program (IKAP) showed that the activity patterns of multiple kinases that regulate SHH pathway (such as PKA, GRK2, P38-MAPK, etc.) were altered in *Scrib*KO cells, suggesting that Scrib has a profound effect on multiple phosphorylation events. Overall, our data here elucidate a more detailed molecular connection between Scrib and the SHH pathway activation: Scrib modulates the phosphorylation of Gli2. These results further support our hypothesis that Scrib is a candidate target against SHH-MB as a potent regulator of the SHH pathway.

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