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The Role of Suppressor of Fused in Development and Tumorigenesis in the Mouse

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ABSTRACT

Embryonic development is a process that involves a number of evolutionarily well-conserved signaling cascades, including the hedgehog pathway. Mutations in components of this pathway have been identified in certain developmental disorders, and in many different kinds of cancers. In fact, the most common cancer in the Western World, basal cell carcinoma (BCC) of the skin, is due to mutations that cause aberrantly activated hedgehog signaling. This thesis focuses on a protein known as Suppressor of fused (Sufu), which is an essential tumor suppressor within the hedgehog pathway. In **PAPER I**, we made the surprising observation that Sufu actually plays a fundamental role in the mammalian hedgehog signaling pathway, in contrast to its role in fruit flies and even zebrafish. In these organisms, Sufu plays an insignificant part in normal hedgehog signaling, since its absence only results in minor phenotypic changes. However, in the mouse, we showed that loss of Sufu leads to embryonic death in mid-gestation with the embryos exhibiting severe cephalic defects and an open neural tube. We also demonstrated that the *Sufu* loss-of-function phenotype was due to ligand-independent activation of the hedgehog signaling pathway.

In humans, Gorlin syndrome is a rare developmental disorder that in the majority of cases is due to inactivating mutations in the gene that encodes the hedgehog receptor, PTCH1. This leads to overactivated hedgehog signaling, since PTCH1 is no longer able to inhibit the signal transducer, Smoothened (SMO). Gorlin syndrome involves an array of different developmental defects, but it also leads to increased tumor susceptibility, especially in the form of multiple BCCs. In PAPER I we discovered that mice, heterozygous for the *Sufu* gene, develop features of Gorlin syndrome, including a skin phenotype with BCC-like attributes, in addition to developmental aberrations in the form of jaw keratocysts. In addition, we showed that the extent of epidermal skin changes correlated with increased hedgehog pathway activation.

The BCC-like lesions in $Sufu^{+/-}$ mice are reminiscent of basaloid follicular hamartomas (BFH), which are more benign lesions than BCCs. In **PAPER II**, the aim was to investigate whether the $Sufu^{+/-}$ skin lesions would develop into full-blown BCCs if *Trp53* was knocked out simultaneously. *Trp53* is a well-known tumor suppressor gene that can enhance hedgehog-driven tumors, and is often mutated in sporadic BCCs, sometimes in combination with *PTCH1* mutations. We showed that $Sufu^{+/-}$ mice on a *Trp53* null background develop medulloblastomas and rhabdomyosarcomas, which is consistent with previous reports. Surprisingly, however, the $Sufu^{+/-}$ skin phenotype was not altered in the absence of *Trp53*, and showed no changes in latency, multiplicity, cellular phenotype or proliferative capacity during the lifespan of the mice. This finding suggests a differential, tissue-specific sensitivity to *Sufu* and *Trp53* gene loss, possibly linked to developmental phase and proliferative potential in specific tissues.

In **PAPER III**, we studied developmental and differentiation processes in the absence of *Sufu*, using embryonic stem cells (ESCs) derived from $Sufu^{-/-}$ pre-implantation embryos. $Sufu^{-/-}$ ESCs were found to express typical pluripotency markers, but the activity of the hedgehog pathway was increased only modestly compared to wild-type ESCs, as indicated by *Gli1* target gene expression. The $Sufu^{-/-}$ ESCs formed embryoid bodies *in vitro*, which, in later stages, were smaller than their wild-type counterparts, suggesting a deficiency in proliferation. To test the differentiation capacity of the $Sufu^{-/-}$ ESCs *in vivo*, the cells were injected subcutaneously into nude mice to form teratomas. Teratomas from $Sufu^{-/-}$ ESCs developed at efficiencies and latencies equivalent to teratomas from wild-type ESCs, yet in stark contrast to wild-type, $Sufu^{-/-}$ teratomas were dominated by neuroectodermal tissues and were deficient in the mesodermal derivatives, cartilage and bone. These findings call attention to the central role played by *Sufu* in the hedgehog signaling pathway, and propose a function for *Sufu* in ectodermal-mesodermal cell fate decision.

In a **PRELIMINARY STUDY**, we have generated conditional *Sufu* mutant mice with the aim of deleting *Sufu* in specific tissues at specific time-points. These studies are ongoing, and experiments to create mice with complete loss of *Sufu* in the K5 (basal cell) compartment of the skin have been initiated.

In summary, the studies in this thesis highlight an essential role for *Sufu* in the hedgehog signaling pathway during development and tumorigenesis in mammals.

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