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TRIB3 suppresses tumorigenesis by controlling mTORC2/AKT/FOXO signaling

María Salazar[1,2,†,‡], Mar Lorente[1,2,†], Elena García-Taboada[1], Eduardo Pérez Gómez[1,3], David Dávila[1,2], Patricia Zúñiga-García[4], Juana M Flores[5], Antonio Rodríguez[5], Zoltan Hegedus[6], David Mosén-Ansorena[4], Ana M Aransay[7], Sonia Hernández-Tiedra[1,2], Israel López-Valero[1,2], Miguel Quintanilla[8], Cristina Sánchez[1,3], Juan L Iovanna[9], Nelson Dusetti[9], Manuel Guzmán[1,10], Sheila E Francis[11], Arkaitz Carracedo[4,12,13], Endre Kiss-Toth[11], and Guillermo Velasco[1,2,*]

[1]Department of Biochemistry and Molecular Biology I; School of Biology; Complutense University; Madrid, Spain; [2]Instituto de Investigaciones Sanitarias San Carlos (IdISSC); Madrid, Spain; [3]Instituto de Investigación Hospital 12 de Octubre (I+12); Madrid, Spain; [4]CIC bioGUNE, Bizkaia Technology Park; Derio, Spain; [5]Department of Animal Surgery and Medicine; School of Veterinary; Complutense University; Madrid, Spain; [6]Institute of Biophysics; Hungarian Academy of Sciences; Szeged, Hungary; [7]CIC bioGUNE-CIBERehd, Bizkaia Technology Park; Derio, Spain; [8]Instituto de Investigaciones Biomédicas Alberto Sols; Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid (CSIC-UAM); Madrid, Spain; [9]Centre de Recherche en Carcérologie de Marseille (CRCM); INSERM UMR 1068, CNRS UMR 7258, Aix Marseille Université and Institut Paoli Calmette; Marseille, France; [10]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED) and Instituto Ramón y Cajal de Investigaciones Sanitarias (IRYCIS); Madrid, Spain; [11]Department of Cardiovascular Science; University of Sheffield; Sheffield, UK; [12]Ikerbasque, Basque Foundation for Science; Bilbao, Spain; [13]Biochemistry and Molecular Biology Department; University of the Basque Country (UPV/EHU); Bilbao, Spain

[†]These authors equally contributed to this work;
[‡]Current address, Cell Division and Cancer Group, Spanish National Cancer Research Center (CNIO), Madrid E-28029, Spain

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In a recent article, we found that Tribbles pseudokinase 3 (TRIB3) plays a tumor suppressor role and that this effect relies on the dysregulation of the phosphorylation of v-akt murine thymoma viral oncogene homolog (AKT) by the mammalian target of rapamycin complex 2 (mTORC2 complex), and the subsequent hyperphosphorylation and inactivation of the transcription factor Forkhead box O3 (FOXO3).

Pseudokinases—a subgroup of protein kinases that lack at least one of the conserved catalytic residues present in the kinase domain and therefore exhibit no (or very low levels of) kinase activity[1]—have been proposed to play critical roles as activators of their specific targets [1]. Likewise, their aberrant regulation has been implicated in the etiology and progression of a variety of diseases, including cancer.[2] Tribbles pseudokinase-3 (TRIB3; also named TRB3, NIPK, and SKIP3), which belongs to the tribbles family of pseudokinases, was first described in *Drosophila* as a negative regulator of cell division in early embryogenesis[1] and has been proposed to interact with different targets including mitogen activated protein kinases (MAPKs) and several transcription factors[3]). TRIB3 has also been shown to interact with and inhibit v-akt murine thymoma viral oncogene homolog (AKT),[4] which has been suggested to suppress insulin signaling.

In our study,[5] we investigated the effect of genetic inactivation of *TRIB3* in several cellular and animal models of cancer. We found that genetic inhibition of *TRIB3* enhances tumorigenesis and that this effect relies to a large extent on the ability of this pseudokinase to regulate activity of the AKT pathway. For example, we found that loss of TRIB3 enhances the frequency of malignant conversion of papillomas generated in mice subjected to 7,12-dimethylbenz[a]anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA) treatment, and that loss of TRIB3 enhances the incidence of premalignant and malignant lesions in phosphatase and tensin homolog heterozygous ($Pten^{+/-}$) mice. Likewise, genetic inhibition of *TRIB3* enhances proliferation, clonogenicity, and the ability to generate tumor xenografts of oncogene-transformed mouse embryonic fibroblasts (MEFs) and of several human cancer cell lines, with a parallel increase in the phosphorylation of AKT in samples derived from these tumors. Together, these findings indicate that genetic inhibition of *TRIB3* enhances tumorigenesis in several genetic contexts and specifically in the presence of activating mutations of rat sarcoma virus oncogene (Hras) or deletion of one of the copies of *Pten*. Nevertheless, other studies have found that *TRIB3* mRNA levels are

*Correspondence to: Guillermo Velasco; Email: gvd@bbm1.ucm.es
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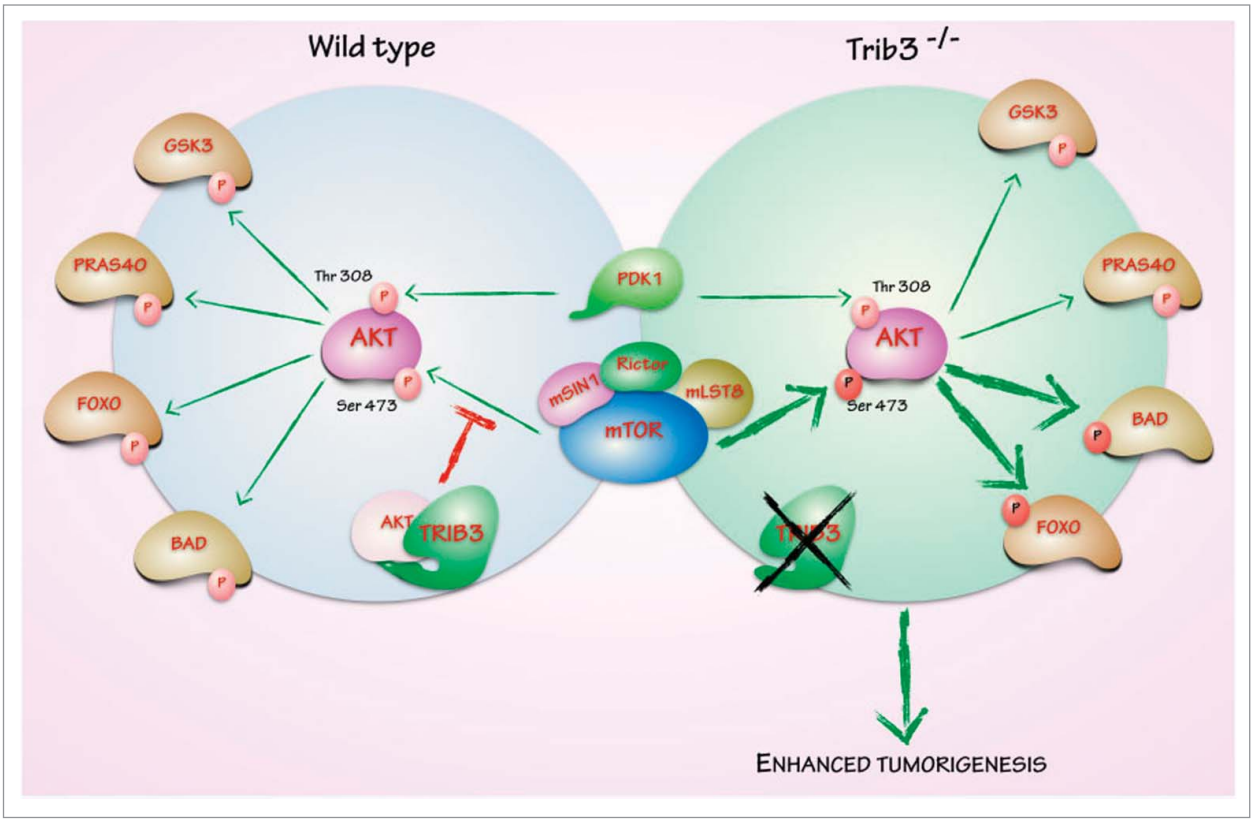

Figure 1. Putative mechanisms by which TRIB3 controls tumorigenesis. Tribble pseudokinase 3 (TRIB3) interacts with AKT, which regulates phosphorylation of the kinase by the mTORC2 complex (wild type). Genetic inhibition of *TRIB3* in combination with different oncogenic signals facilitates hyperphosphorylation of AKT on Ser 473 by the mammalian target of rapamycin complex 2 (mTORC2 complex) and the subsequent hyperphosphorylation and inactivation of the transcription factor Forkhead box O3 (FOXO3) and the BH3-only protein BCL2-associated agonist of cell death (BAD), but not that of other AKT downstream targets. The hyperphosphorylation and inactivation of FOXO is, at least in part, responsible for the enhanced tumorigenic features of TRIB3-deficient cells.

increased in certain types of human cancer[2,6] and have proposed that TRIB3 may play an oncogenic role.[7] Further research should clarify whether inactivation or enhanced expression of TRIB3 produces different outcomes in distinct genetic or cellular contexts.

Our findings indicate that the tumor suppressive activity of TRIB3 relies on its ability to limit the capacity of AKT to become overactivated in response to oncogenic signals. We found that genetic inactivation of *TRIB3* leads to enhanced phosphorylation of Forkhead box O3 (FOXO3) and BCL2-associated agonist of cell death (BAD), but not of other AKT substrates such as glycogen synthase kinase 3 (GSK3) or AKT1 substrate 1 (AKT1S1; also named proline rich AKT substrate or PRAS40), suggesting that TRIB3 contributes to the regulation of AKT selectivity for some of its substrates. In line with this idea, we have recently found that treatment with Δ^9-tetrahydrocannabinol (THC, a compound derived from the plant *Cannabis sativa* that exerts antitumor effects in mouse models of cancer[8,9]) triggers AKT inhibition via enhanced interaction of TRIB3 with AKT and a subsequent decrease in the interaction of AKT and TRIB3 with the mTORC2 complex.[10] These observations indicate that interaction with TRIB3 negatively regulates AKT by restricting access of the kinase to the mammalian target of rapamycin complex 2 (mTORC2 complex) and that, through this mechanism, TRIB3 contributes to the regulation of AKT selectivity for some of its substrates (**Fig. 1**).

Another conclusion of our work is that the mechanism underlying the tumor suppressive activity of TRIB3 downstream of AKT relies at least in part on the regulation of FOXO3 activity. In support of this idea, we found that re-expression of a mutant form of FOXO3 in which the residues phosphorylated by AKT have been mutated to Ala (FOXO-A3) abolished the enhanced proliferation and clonogenicity of TRIB3-deficient cells and decreased the proliferation and growth rate of tumors generated with these cells. These observations support that FOXO3 inactivation plays a crucial role in the enhanced tumorigenic features of cells in which Trib3 is genetically inactivated. Nevertheless, expression of the FOXO-A3 mutant did not modify the time to occurrence of tumors derived from TRIB3-deficient cells suggesting that, together with the regulation of FOXO activity, TRIB3 may use additional mechanisms to control tumorigenesis.

In summary, our findings show that genetic inhibition of *TRIB3* increases

tumorigenesis in several animal models of cancer and that this effect is due, at least in part[AQ3], to enhanced phosphorylation of AKT by the mTORC2 complex and subsequent hyperphosphorylation and inactivation of FOXO3.

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