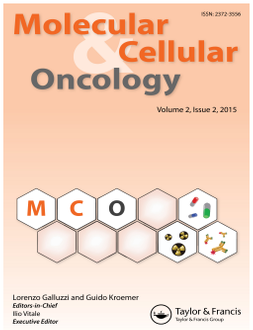

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AUTHOR'S VIEWS

From mouse genetics to targeting the Rag GTPase pathway

Ana Ortega-Molina and Alejo Efeyan

Metabolism and Cell Signaling Laboratory, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

ABSTRACT

The identification of the Rag GTPases initiated the deciphering of the molecular puzzle of nutrient signaling to the mechanistic target of rapamycin (mTOR), and spurred interest in targeting this pathway to combat human disease. Recent mouse genetic studies have provided pathophysiological insight and pointed to potential indications for inhibitors of the Rag GTPase pathway.

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Evolution has shaped the ability of cells to adjust metabolism to the availability of nutrients and energy, and fundamental nutrient-sensing mechanisms have been tuned to preserve nutrients during scarcity, and to allow growth and storage during periods of surplus. Among these mechanisms, the signaling pathway of the Rag guanosine tri-phosphatases (GTPases; known as the Rag GTPases) upstream of mechanistic target of rapamycin complex 1 (mTORC1) detects and signals a repertoire of nutrients (amino acids, glycolytic intermediates, lipids) to couple the anabolic processes controlled by mTORC1 to nutrient sufficiency. Upon nutrient sufficiency, the heterodimeric RagA/RagC complex recruits mTORC1 to the outer lysosomal surface, the subcellular location where mTORC1 can then undergo kinase activation in a growth factor-dependent manner. Thus, both nutrient-signaling (recruitment) and growth factor-signaling (kinase activation) are essential inputs for the regulation of mTORC1.[1]

Within our efforts to establish the impact of nutrient signaling in human health and disease, we generated gain- and loss-of-function alleles for three Rag GTPase genes (*Rraga, Rragb* and *Rragc*, known as RagA, RagB and RagC, respectively) in mice (Figure 1a).[2] Genetic deletion of the Rag heterodimeric complex resulted incompatible with rapid cellular proliferation, while mice endogenously expressing a constitutively active version of RagA ($Raga^{Q66L}$) taught us that physiological fluctuations in nutrient – Rag GTPase signaling are critical to control glycemia and to trigger metabolic adaptation to fasting in neonates and in adult mice (Figure 1a).[3–6] The latter findings support that the suppression of the Rag GTPase pathway may mirror aspects of nutrient limitation, and argue that pharmacological attenuation of this pathway could result in some of the benefits of dietary restriction, a concept with genetic support in lower organisms, and consistent with the pro-longevity effects of the mTORC1 inhibitor rapamycin.[7]

But most unexpected and prominent were the detrimental effects of genetic manipulation of the Rag GTPases on B lymphocyte functions: genetic deletion of *Raga* impaired B cell development, while expression of the constitutively-active $Raga^{Q66L}$ rendered mature B cells with impaired ability to form anatomical structures key for B cell proliferation and antibody maturation, called germinal centers (GC), during the humoral response (Figure 1a).[3,8]

In parallel to this work, recurrent, point, activating mutations in the nucleotide binding domain of RagC (*RRAGC* gene in humans) were observed in around 15% of follicular lymphoma (FL) patients.[9] FL is an indolent and incurable B lymphoid malignancy that arises as a pathological outcome of physiological, extensive GC B-cell proliferation coupled to mutagenesis, resulting in the emergence of pro-oncogenic genetic lesions. $RAGC^{T90N}$ and $RAGC^{S75C}$ mutations (found in different FL patients) were independently knocked-in in the *Rragc* locus in mice (to encode amino acid substitutions T89N and S74C), to decipher the oncogenicity of nutrient signaling in B cells. Expression of RagC mutant variants caused partial insensitivity to nutrient deprivation, a mild increase in the nutrient-mTORC1 signaling axis, and strongly exacerbated B cell responses including enhanced GC formation, class-switch recombination, and plasma cell production (Figure 1a). In addition, expression of $RagC^{S74C}$ or $RagC^{T89N}$, in cooperation with *Bcl2* overexpression (*BCL2* is translocated in 90% FL), accelerated lymphomagenesis and created a selective vulnerability to rapamycin.[10] Taken together, these studies in B cells show that only a mild activation of the Rag GTPases confers an intrinsic selective advantage, while a complete insensitivity to nutrient deprivation results deleterious. The underpinnings for such a prominent importance of Rag GTPase signaling in B cells is not clear, but is likely to be related with 1) the minimal cytoplasmic content of resting B cells, and 2) the need for a sudden anabolic and proliferative burst upon activation of the humoral response, thus requiring a tight control of the intrinsic anabolic capacity to endure and accomplish such onerous burst.

Collectively, mouse genetics and clinical data point to a potential use of nutrient signaling inhibitors against FL. But such small molecules are still under development, so to assess the impact of a partial suppression of nutrient signaling *in vivo*, which

CONTACT Alejo Efeyan aefeyan@cnio.es Metabolism and Cell Signaling Laboratory. Spanish National Cancer Research Centre (CNIO), Madrid, Spain

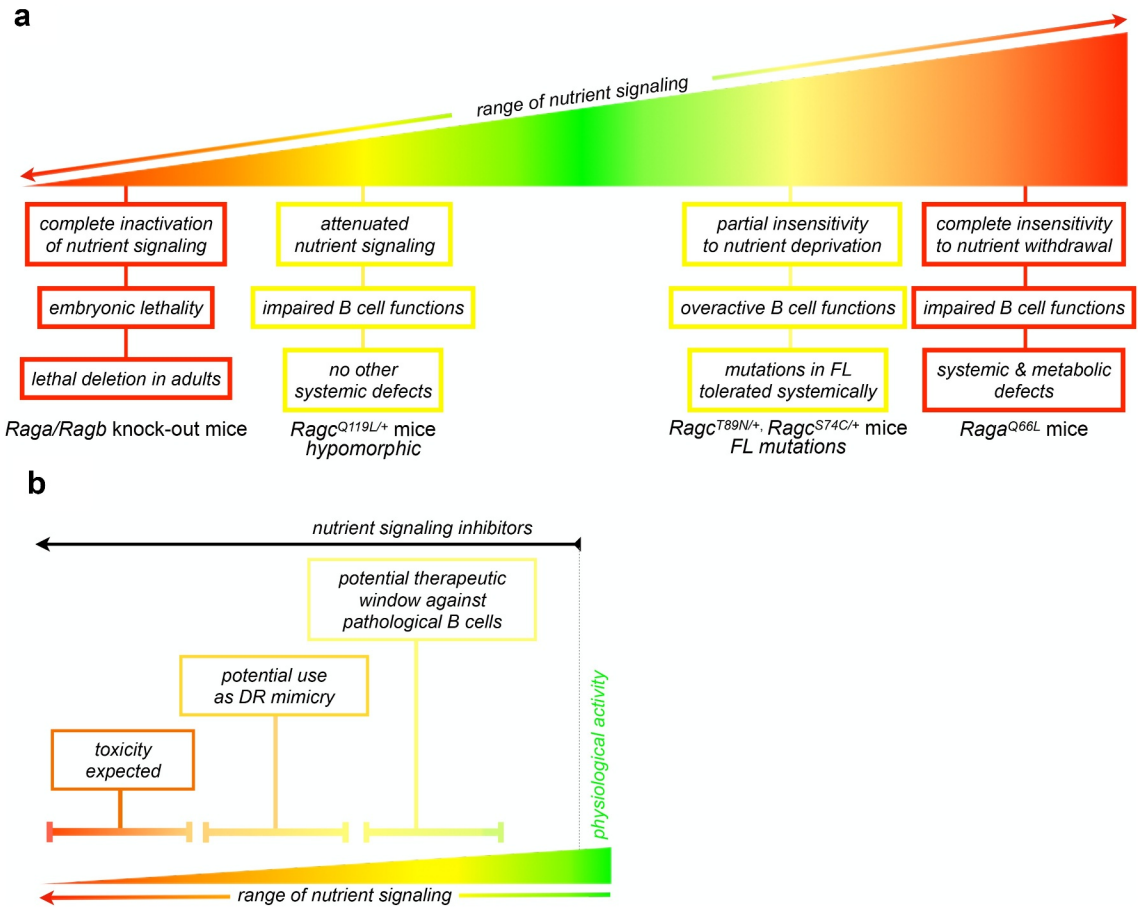

Figure 1. Genetic manipulation of the Rag GTPases in mice and the expected consequences of pharmacological targeting of Rag GTPase signaling. (a). Consequences genetic activation and inhibition of the Rag GTPases in mice. The triangle depicts the range of Rag GTPase signaling *in vivo*. Phenotypes observed using the gain- and loss-of-function alleles for *Raga* and *Ragc* are depicted in colored rectangles. (b). Expected, potential effects of Rag GTPase signaling inhibitors, as inferred by mouse genetics. Milder inhibitors may selectively target B cell functions, inhibitors of intermediate potency may partially suppress nutrient signaling in most organs, mimicking features of dietary restriction (DR). Finally, close-to-complete inhibition of Rag GTPase signaling is likely to cause deleterious effects.

would typically mirror the incomplete effect of pharmacological inhibitors, we undertook a genetic approach. We used CRISPR-Cas9 genome editing to endogenously express an inactive variant of RagC ($RagC^{Q119L}$) in mice. $Ragc^{Q119L/+}$ mice showed attenuated mTORC1 activity, a strikingly impaired B cell activation upon immunization, and delayed development of FL. The strong suppressive effect on B cells was in sharp contrast with the lack of detrimental consequences on health and survival of $Ragc^{Q119L/+}$ mice, pointing to an exquisite sensitivity of B cells to a subtle inhibition of nutrient signaling, and thus to the existence of a therapeutic window upon Rag GTPase inhibition that could selectively affect B cells, sparing negative effects in other cell types, thus accomplishing safety and efficacy (Figure 1a).[11]

But the lack of systemic effects by attenuation of nutrient signaling (including normal longevity of $Ragc^{Q119L/+}$ mice) argues that this pathway may not mediate beneficial metabolic effects of nutrient limitation. We do not favor this statement, and instead propose that the limited extent of inhibition of nutrient signaling by heterozygous expression of the Q119L allele is enough to suppress the exquisitely sensitive B cell activities, but insufficient to exert the beneficial effects of mTORC1 inhibition in other organs (Figure 1b). Stronger, albeit incomplete, pharmacological inhibition of nutrient signaling may suppress the Rag GTPase – mTORC1 axis to an extent that causes systemic reduction of anabolic metabolism, and in (some of) the benefits of reduced nutrient intake. In other words, we believe that mild pharmacological inhibition of Rag GTPase signaling may be efficacious and safe against pathological B cells, while stronger, albeit still incomplete inhibition, may be required to unleash systemic metabolic effects in other organs, including aspects of dietary restriction (DR) mimicry. Upon increasing power of Rag GTPase signaling inhibitors, it is reasonable to expect widespread toxicities (Figure 1b), as the phenotyping of *Rraga*/*Rragb* knock-out mice has shown. Most of these conclusions are inferred from mouse genetics tools and from work in other model organisms, but nonetheless provide a framework for anticipating what to expect and not to expect from Rag GTPase signaling inhibitors, and what indications we will need to explore.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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ORCID

Ana Ortega-Molina http://orcid.org/0000-0003-2782-8994
Alejo Efeyan http://orcid.org/0000-0002-3806-6799

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