CANCER METABOLISM

Losing control of nutrient sensing in the germinal centre drives lymphomagenesis

The gene encoding the RagC GTPase (*RRAGC*), an activator of a nutrient-sensing pathway that drives cellular anabolism, is mutated in 15% of follicular lymphoma cases. A new study provides evidence that *RRAGC* mutations promote lymphomagenesis by distorting the nutrient-dependent control of paracrine signals from the microenvironment, thus enhancing B-cell activation.

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Follicular lymphoma (FL) is an indolent but largely incurable non-Hodgkin's lymphoma originating from the clonal expansion of germinal centre (GC) B cells[1], a specialized subset of B cells that, during a T-cell-dependent immune response, modify their rearranged immunoglobulin variable-region genes, thus generating highly specific pathogen-eliminating antibodies[2,3]. The genetic hallmarks of FL are the t(14;18) chromosomal translocation, which constitutively activates the anti-apoptotic gene *BCL2*, and recurrent mutations of histone-modifier genes[1]. Additional genetic mutations affecting various biological pathways have been identified by genomic analyses[1,4], including aberrations in components of the cellular nutrient-sensing pathway. Specifically, a substantial subset of FL cases show missense mutations in the nucleotide-binding domain of the gene encoding the RagC GTPase (*RRAGC*)[5,6]. This enzyme forms a heterodimeric complex together with the RagA GTPase, which activates mammalian target of rapamycin complex 1 (mTORC1) when cellular nutrients are sufficient, thus leading to cell growth[7,8]. Curiously, RagA, despite being part of the mTORC1-activating complex, is not targeted by mutations in FL[5,6]. These observations have prompted two questions: how do mutations in *RRAGC* promote FL pathogenesis, and what is the basis for the selectivity of mutations in *RRAGC*, sparing RagA? Through mimicking the FL-associated *RRAGC* mutations in the mouse germline and crossing those mice to an established FL animal model, Ortega-Molina et al. have now uncovered a role for RagC in normal GC B-cell development and FL pathogenesis[9].

Using CRISPR–Cas9 genome engineering, Ortega-Molina et al. generated two independent *Rragc*-knock-in mouse models mimicking the most frequent human variants of activating *RRAGC* mutations[9]. To assess the presumed aberrant activity of mutated RagC in vitro, the authors omitted amino acids and growth factors that would elicit mTORC1 activation from the culture medium. Under these conditions, B cells from the mutant mouse lines showed greater mTORC1 activity than those from wild-type mice after T-cell-mediated signalling that stimulates the mTORC1 pathway via the PI3K–AKT axis during the immune response (Fig. 1a); these results suggest a partial insensitivity to nutrient withdrawal (Fig. 1b). The same response has been observed on a *Bcl2*-transgenic background in *VavP-Bcl2* transgenic mice[10], thus also revealing accelerated FL development in vivo[9]. Transcriptional profiling analysis showed enrichment of the mTORC1 signature in RagC-mutated FL cells and a marked overlap in the differential expression of the corresponding genes in *RRAGC*-mutated mice and human FL. These results suggest that RagC-mediated enhancement of mTORC1 activation also promotes FL pathogenesis in humans.

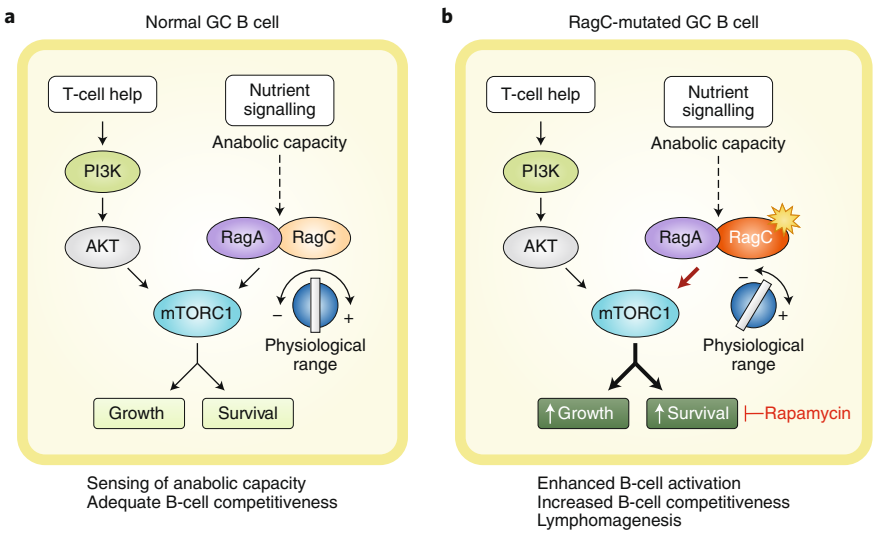

Fig. 1 | Model of the effects of RagC mutations on GC B-cell development and lymphomagenesis. a, The nutrient-signalling pathway imposes an 'anabolic capacity' barrier over B-cell activation by T-cell help (and B-cell-receptor signalling; not shown), thus ensuring that growth occurs only if nutrients are sufficient. GC B cells are clonally selected for the expression of high-affinity antibodies (adequate B-cell competitiveness). **b**, RagC mutations weaken this barrier and cause enhanced B-cell activation while retaining the ability to suppress activation when nutrient levels are low. These mutations lead to increased B-cell competitiveness, which translates into enhanced B-cell growth and survival. After acquiring additional transforming events, GC B cells ultimately develop into lymphomas. RagC-mutated cells are sensitive to rapamycin-mediated pharmacological inhibition of mTORC1. Figure adapted from ref. [9], Springer Nature Limited.

To gain insights into the pathogenic mechanism of mutated RagC in FL development, the authors determined the biological effects of the mutated gene in the normal cellular counterpart of FL. After immunizing mice with a T-cell-dependent antigen, the authors observed a dramatically higher abundance of GC B cells in RagC-mutated mice than in wild-type controls[9]. The difference was even more marked in a competitive reconstitution setting in which the ability of RagC-mutant and wild-type B cells to generate GCs in the same mouse was assessed. Mechanistically, the authors provide evidence that this increase in RagC mutant cells is likely to be due to suppression of cell death and to a decreased requirement of RagC-mutated GC B cells on microenvironmental signals provided by T-follicular helper (T_{FH}) cells, the $CD4^+$ T cells that control the GC reaction. The latter was evident from the observation that, despite the massive enlargement of GCs in the RagC-mutated mice, the number of T_{FH} cells per GC was similar to that in control mice. Because T_{FH} cells are required for GC maintenance, the decrease in the T_{FH}/GC B-cell ratio suggests that *Rragc* mutations promote lymphomagenesis by substituting PI3K–AKT-derived mTORC1-activating signals through the Rag GTPase–mediated nutrient-sensing pathway (Fig. 1b).

Now, if aberrant Rag GTPase function promotes FL pathogenesis via mTORC1 activation, why are no activating mutations observed in RagC's heterodimeric partner RagA? Ortega-Molina et al. provide evidence for what they call the 'biochemical asymmetry' of Rag heterodimers in the activation of mTORC1. In contrast to the observation in which mutated RagC was sensitive to nutrient withdrawal, an activating mutation in RagA had no effect on the regulation of mTORC1 under the same condition[9]. Moreover, the RagA mutation did not lead to an enlargement of GCs, and constitutive activation of RagA in GC B cells actually impaired the GC response by decreasing GC B-cell fitness, similarly to previously reported observations for mTORC1 hyperactivation[11]. Specifically, strong mTORC1 activation negatively affected the generation of high-affinity antibodies against the immunizing antigen, a response that, in a competitive setting, has been found to lead to the disappearance of mTORC1-hyperactive versus wild-type GC B cells over time[11]. In contrast, mutations in RagC have been found to increase the production of high-affinity antibodies[8]. On the basis of these findings, the authors propose a model in which the mutations in RagC, as opposed to those in RagA, lead to a modest activation of the mTORC1 pathway that increases GC B-cell fitness (Fig. 1b).

But how would increased fitness of RagC-mutated GC B cells contribute to the multistep process of FL development? Adding to the pro-survival signals provided by *Bcl2* translocation, RagC-induced mTORC1 activation may further suppress the default apoptotic program of GC B cells by enhancing activating T_{FH}-cell-derived signals, which also funnel into the mTORC pathway. These pre-malignant GC B cells have a competitive advantage over normal GC B cells with similar antigen affinities and continue to undergo iterative cycles of selection and proliferation within the GC, during which additional genetic aberrations may be acquired, and bone fide lymphomas may ultimately develop.

Then the pressing question is whether enhanced mTORC1 activity in FL with *Rragc* mutations and *Bcl2* translocations might be exploited for lymphoma therapy. Experiments performed by Ortega-Molina et al. with the mTOR inhibitor rapamycin indicated that such mice, in contrast to mice with only *Bcl2* translocations, showed a lower incidence and grade of lymphomas[9]. The greater selective sensitivity to mTORC1 inhibition observed in this mouse model suggests that patients with mutations in the nutrient-signalling pathway may benefit from treatment with mTOR inhibitors.

Finally, the authors propose that a different genetic aberration in FL may act in a similar manner to *RRAGC* mutations. Loss-of-function mutations or deletions of *TNFRSF14* that ablate the function of herpes virus entry mediator are associated with an increased cellularity of T_{FH} cells in the tumour microenvironment[12]. A meta-analysis of genomic data from human FL samples has revealed that *TNFRSF14* mutations and *RRAGC* mutations are largely mutually exclusive[9]. Intriguingly, a recent publication has reported that a deficiency in herpes virus entry mediator is associated with increased B-cell competitiveness during the GC reaction[13], thus mirroring observations by Ortega-Molina et al. in RagC-mutated B cells. Therefore, T_{FH}-cell-derived signals may promote lymphoma growth either by cell-autonomous mutations that synergize with those signals, as observed for *RRAGC* mutations, or by abnormally high T_{FH}-cell numbers, as in the case of *TNFRSF14* deficiency. The findings from Ortega-Molina et al. provide important new insights into the molecular mechanisms of FL pathogenesis. In future studies, the potential tumour metabolic vulnerability identified in the present work may be exploited for the development of precision-medicine-based therapies. ❐

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Competing interests

The authors declare no competing interests.