

Image: Fotolia/Andrea Danti

Modulation of T-cell development in Thessaloniki, Greece

# Whose Loss Leaves the Immune System Imperilled

Only recently, an immunomodulatory role for the 'cylindromatosis' tumour suppressor gene (Cyld) has been ascribed. In their pioneering work, George Mosialos and his group have cracked the mechanism of Cyld-dependent regulation of T-cell development with their *in vivo* mouse model. Excerpts from a tête-à-tête with Mosialos follow...

The life and health of a cell are dictated by precisely modulated pathways of protein synthesis and degradation. Among these modulatory mechanisms are the extrinsic factors that operate in the cellular environment and the intrinsic pathways that direct the cell from within.

Post-translational modification (PTM) of proteins represents one of the many cell-autonomous processes of protein regulation that are crucial to the pathophysiology of a cell. The activity of one of the first tumour suppressors to be identified, the Retinoblastoma protein, was, in fact, shown to be modulated by phosphorylation. Likewise, deregulated modification of sugar residues in cell surface glycoproteins disrupts adhesion to basal lamina and results in tumour invasion. Many reports have since investigated the role of a whole plethora of protein modifications right from phosphorylation and acetylation to ubiquitination and sumoylation in health and disease.

For over two decades, the research interests of George Mosialos and his colleagues have revolved around the molecular mechanisms of carcinogenesis with a more recent focus on the role of the deubiquitinase cylindromatosis tumour suppressor (Cyld) in T-cell development and leukemogenesis. In their latest paper (*J Immunol.*,185(4):2032-43), the Mosialos group has uncovered fundamental mechanistic aspects of regulation of T-cell development by CYLD using conditional gene targeting in mice. Their work opens portals to understanding the etiology of T-cell malignancies.

## CYLD in cylindromatosis

Following his PhD in Boston in the early 90s, George Mosialos spent many of his post-doctoral years studying the contribution of the tumour virus, Epstein-Barr (EBV), to the progression of human malignancies. What came as a breakthrough was the identification of the mechanism of

EBV-induced Latent Membrane Protein 1 (LMP1)-dependent activation of NFκB signalling in resting B lymphocytes, a process that triggers their indefinite proliferation in tumorigenesis (*Mol Cell Biol.*,19(8):5759-67). Soon, George Mosialos established an independent group of his own at the Alexander Fleming Biomedical Sciences Research Center, in Vari (about 15 km south of Athens) and there pondered the mechanistic details of the NFκB activation pathway in carcinogenesis.

## CYLD as an immune modulator

"We found CYLD in a yeast two-hybrid screen using NEMO, the NFκB essential modulator, as a 'bait' to screen a B cell cDNA library," recalls George. It turned out that CYLD is a deubiquitinating enzyme that negatively regulates the transcriptional activity of NFκB by targetting specific mediators of tumour necrosis factor receptor

Reports suggesting a role for CYLD in immune modulation besides tumorigenesis steered his attention to the function of CYLD in T-cell development. George recalls, "There was increasing evidence of an association of CYLD with immune disorders like inflammatory bowel disease," he continues, "today we know that CYLD is enriched in the thymus, neurons and testes. Its downregulation has been observed in several instances of cancer, including cancers of the colon and cervix and in melanomas and multiple myelomas." In the past, studies of CYLD-deficient mice have shown that the protein is indispensable in T-cell development but have failed to identify the stage at which T-cell development is wrecked. "It is known from previous work that the downregulation of NFκB is a critical step in the differentiation of immature double-positive (DP) thymocytes to mature single-positive (SP) T-cells. To look closer into the CYLD-



From left to right: George Mosialos, Vassilis Mavromatidis, Ageliki Tsagaratou, Paul Hadweh, Dimitris Karatzas, Evi Hatzivassiliou.

(TNFR) signalling (*Nature*, 424(6950):793-6). CYLD mutants that lacked deubiquitinating activity were associated with familial cylindromatosis (tumours of skin adnexa) and hence, their discovery implicated CYLD-dependent regulation of NFκB in cellular homeostasis of skin appendages.

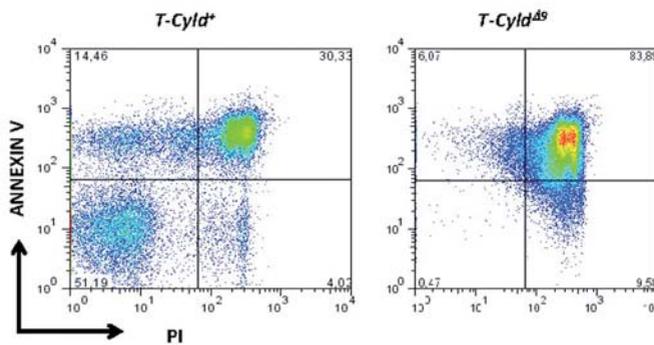
NFκB pathway, we generated a mouse line with T-cell-restricted mutation in the *Cyld* locus engineered to encode a functionally impaired protein," George summarises.

In their recent *Journal of Immunology* paper, Tsagaratou *et al.* have identified for the first time an *in vivo* link between CYLD

and NEMO in the regulation of T-cell development. The group showed that the deficiency of CYLD in the conditional mutants (CYLD<sup>Δ9</sup>) affected the maturation of DP thymocytes to SP T-cells. "In our analyses of thymocyte populations, we followed the expression of the conventional cell surface markers, CD4 and CD8 and of the transient

at the same stage as seen in the CYLD-deficient mice that we've had," says George. The Mosialos lab's studies concur with the findings of other research groups. Downregulation of CYLD by an oncogene of T-cells, Notch, has been associated with the retention of thymocytes in the undifferentiated DP stage predisposing to T-cell

leukaemia. Thus, one can appreciate that the work on CYLD in thymocyte positive selection holds great prospects for a detailed understanding of the development of relevant autoimmune diseases and cancer.



FACS analysis demonstrating the increased susceptibility of CYLD-deficient thymocytes (T-Cyld<sup>Δ9</sup>) to death (visualised by Annexin V and propidium iodide (PI) staining) compared to control thymocytes (T-Cyld<sup>+</sup>)

markers such as CD69 and CD5 and quantified the different populations with FACS," George elaborates. The Mosialos group also observed a striking activation of both NFκB and JNK pathways in the CYLD-defective thymocytes. "Given that components of the JNK and p38 MAPK pathways also serve as substrates of CYLD, we could not exclude these from our study," explains George, as he reiterates the preliminary results of their experiments. But what became a Eureka moment was the result of the intercross between the CYLD<sup>Δ9</sup> and a floxed NEMO line. Inhibition of the NFκB pathway by NEMO deficiency in the double mutants rescued the T-cell development defects associated with CYLD deficiency, bringing down NFκB but not JNK activity to physiological levels. Taken together, the pioneering work of the Mosialos lab showed that CYLD interferes with NEMO function and operates in a linear pathway to fine-tune the activity of downstream NFκB, enabling the differentiation of thymocytes.

### CYLD at the outset

The mechanism of action of CYLD, as decrypted by the Mosialos group may have profound implications. For one, a recent work has established that a T-cell malignancy, T-cell Acute Lymphocytic Leukemia (TALL) correlates with a reduction in CYLD levels and is associated with aberrant T-cell development (*Cancer Cell*, 18(3):268-81). "Latest research has shown that in TALL, the block in T-cell development is precisely

at the same stage as seen in the CYLD-deficient mice that we've had," says George. The Mosialos lab's studies concur with the findings of other research groups. Downregulation of CYLD by an oncogene of T-cells, Notch, has been associated with the retention of thymocytes in the undifferentiated DP stage predisposing to T-cell

### "Our Checklist"

Talking about plans for the future, Mosialos and co. have a well-defined agenda. Speaking on behalf

of his colleagues, George emphasises the need for a comprehensive study of the biochemical events that play a pivotal role in the CYLD-dependent regulation of thymocyte development. In addition, they have begun gene expression profile experiments in the hope of identifying novel gene targets of NFκB in thymocyte development. "We wish to screen for downstream genes that may be deregulated to produce the phenotype that we see. Our results may also provide valuable clues on additional aspects of thymocyte selection or help identify candidates in lineage commitment," continues George. In effect, the lab hopes to achieve a better understanding of the differentiation of T-cells and eventually to apply their results to address T-cell leukemogenesis.

The Mosialos group is presently based at the School of Biology, University of Thessaloniki, Greece. In a competition-friendly setting and with current and former financial support from the European Union, the Greek Ministry of Education and the United States Leukemia and Lymphoma Society, members of the Mosialos lab have been coming up trumps in immunology and cancer research. Assistant Professor, George Mosialos, re-discovers science every day, "I believe in having an open mind before any scientific interpretation. It helps to have a fresh start each time and not be preoccupied by previous reports. This applies even to subjects that have been thoroughly investigated," he concludes, wisely.

MADHUVANTHI KANNAN