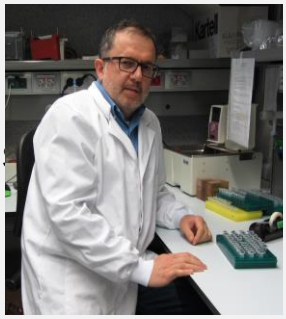
ImmunoTools *special* Award 2014



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Characterization of the role of the proapoptotic protein Cyclin O in haematopoiesis.

The apoptosis or programmed cell death is a physiological process involved in the regulation of the number of cells of an organism. A defective apoptosis is directly related with perturbations in the normal development of the organism, with the tumorogenesis and with the antineoplastic therapy. The chemotherapeutic and physical (ionizing radiations) agents used in the fight against neoplastic diseases use the normal cellular mechanisms of apoptosis for their actions. This fact establishes a clear relationship between the tumour development and the intrinsic resistance to the antineoplastic treatment. In spite of their apparently opposite roles, the processes of cell death, division and differentiation share similarities that are reflected at the molecular level in the use of common biochemical steps. Among them, activation of Cyclin dependent kinases (Cdks) has been shown to be crucial both for mitosis and apoptosis. The activation of the protein kinase Cdk2, originally involved in cell cycle regulation, is necessary for apoptosis of non-dividing cells like thymocytes and neurons. It is controlled by regulatory proteins such as p53, Bcl2 or Bax and it is specific since other members of the Cdk family such as Cdk1 are not activated in the same conditions (Gil-Gómez *et al.*, 1998, *EMBO J.* **17**: 7209-7218 .

Cdk2 activation during apoptosis is dependent on the expression of a new member of the cyclin family, Cyclin O, that will activate the kinase only during cell death (Roig/Roset *et al.*, 2009 *Cell Death Diff* **16**: 230-243). Biochemically, activation of Cyclin O/Cdk2 complexes is a very early step in the apoptosis signal transduction pathway, preceding the activation of apical caspases, the mitocondrial dysfunction and the exposure of phosphatidylserine. Cyclin O expression is necessary for DNA damage and glucocorticoid-induced apoptosis in lymphoid cells but not for apoptosis induced by extrinsic stimuli such as TNFα or Fas/CD95(Granes *et al.*, 2004, *Eur J Immunol.* **10**: 2781-2790).

We have recently generated Cyclin O deficient mice. O. These mice present a complex phenotype characterized by showing perturbations in physiological processes that normally require activation of caspases in both apoptosis and non-apoptosis contexts.

For instance, Cyclin O KO fibroblasts and activated T-cells are profoundly radioresistant. On the other hand, Cyclin O knockout mice develop autoantibodies directed against DNA and antigens present in the basal membrane of the skin, resulting in the development of severe ulcerative blistering dermatitis. This could be related to perturbations in lymphocyte selection and may be related to the action of Cyclin O as a Cdk2 activator (Williams *et al.*, 2000 *Eur J Immunol.* **30**: 709-713) and as an upstream activator of different caspases (Granes *et al.*, 2004, op.cit.).

On the other hand, most of the Cyclin O homozygous knockout mice die within the first month of life because of the development of hydrocephalus.

Haematopoiesis is perturbed by the deletion of the Cyclin O gene. Cyclin O knockout mice are anaemic and thrombocytopenic and show a highly decreased spleen cellularity. The myeloid compartment is also affected.

The defective erythro and thrombopoiesis may likely be a consequence of the requirement of executory caspases (Droin *et al.*, 2009 *Front Biosci* **14**: 2358-71).

To further investigate this we plan to set up *in vitro* culture systems that recapitulate the differentiation of erythroid precursors to mature erythrocytes (Shuga *et al.*, 2007 *Proc Natl Acad Sci U.S.A.* **104**: 8737-8742), megakaryocytes to platelets (Matsubara *et al.*, *PLoS One.* 2013 **8**:e58123) and myeloid and lymphoid cells (Redecke *et al.*, 2013, *Nat Methods.* **10**: 795-803) starting from bone marrow cells obtained from Cyclin O WT and KO mice. By studying the development of the different blood cell linages using these defined culture systems we expect to identify which steps are affected by the lack of Cyclin O. Their flexibility allows them also to be used to differentiate haematopoietic stem cells or even ES cells.

We expect then, to gain knowledge about of the role of Cyclin O in the development of the haematopoietic system and its role in caspase dependent and independent processes.