

Tumour Suppression Group

Summary

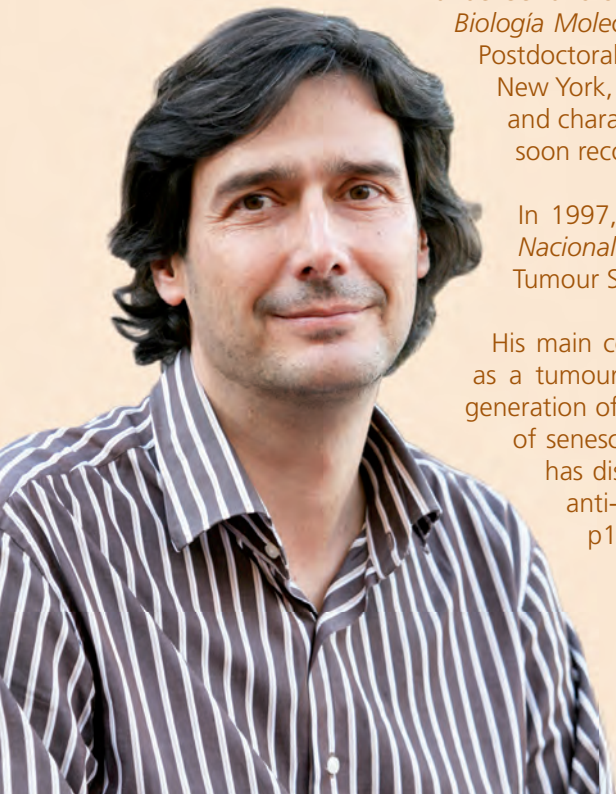
Tumour suppressors are genes that can prevent the development of cancer. Despite their efficient protection against cancer, with time these genes may become defective either accidentally or through the action of mutations. In this manner, the affected cells become partially unprotected from cancer and additional mutations in other genes may give rise to the full development of cancer.

Understanding how tumour suppressor genes work may help design drugs that block cancer growth. We manipulate the mouse genome to create novel alterations that increase or decrease tumour suppression potency.

Strategic Goals

- Understand the mechanisms of tumour suppression
- Study the interplay between tumour suppression and ageing
- Characterise the relevance of cellular senescence in cancer
- Identify new regulators of tumour suppression

Manuel Serrano *Group Leader*



Manuel Serrano obtained his PhD in 1991 for his research on DNA replication at the *Centro de Biología Molecular* under the supervision of M. Salas. From 1992 to 1996 he worked as a Postdoctoral Fellow in the laboratory of D. Beach at the Cold Spring Harbor Laboratory, New York, USA. During this time, he made his most important discovery with the cloning and characterisation of p16, which defined a new class of cell cycle regulators and was soon recognised as a key tumour suppressor.

In 1997, Serrano returned to Spain to start his own research group at the *Centro Nacional de Biotecnología*, in Madrid. He moved to the CNIO in 2003 to lead the Tumour Suppression Group.

His main contributions relate to the concept of oncogene-induced cellular senescence as a tumour suppression mechanism, the role of p19Arf as an oncogenic sensor, the generation of novel mouse models with increased cancer resistance, and the identification of senescent tumour cells within premalignant tumours. Most recently, his laboratory has discovered a *cis*-regulatory element at the *p16* and *p19Arf* locus, reported the anti-ageing activity of the Arf/p53 module, and characterised the role of p16 and p19Arf during reprogramming.

He has received the FEBS Anniversary Prize of the *Gesellschaft für Biochemie und Molekularbiologie*, the Carcinogenesis Young Investigator Award, the *Fundación Echevarne* National Award in Oncology, the *Fundación Banco de Sabadell* National Award in Biomedical Research, and the *Fundación Carmen y Severo Ochoa* Award. Manuel Serrano is an elected EMBO Member.



Staff scientists: Manuel Collado, Luis E. Donate, Susana Llanos, Antonio Maraver, Cristina Pantoja, Susana Velasco. **Post-doctoral fellows:** María Abad (since May), Han Li Sandrina Nobrega (since April), Daniela Piazzolla. **Graduate students:** Pablo J. Fernández-Marcos (until September), Daniel Herranz, Lucía Morgado (since October), Ana Ortega Adelaida Palla (since July), Aránzazu Villasante. **Technician:** M. Isabel Muñoz.

Highlights

The *Ink4/Arf* locus is a barrier for induced pluripotent stem (iPS) cell reprogramming

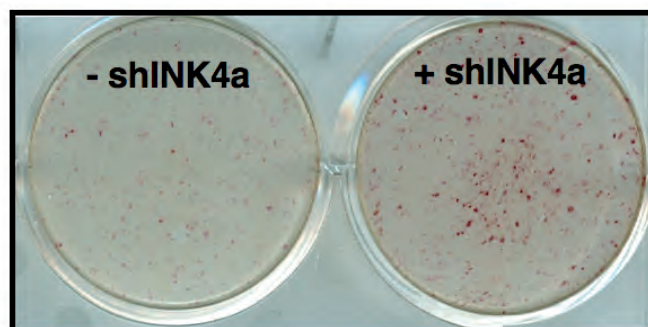
The mechanisms involved in the reprogramming of differentiated cells into induced pluripotent stem (iPS) cells by the three transcription factors Oct4, Klf4 and Sox2 is poorly understood. The *Ink4/Arf* locus encodes three potent tumour suppressors, namely p15^{Ink4b}, p16^{Ink4a} and p19^{Arf}, which are basally expressed in differentiated cells and up-regulated by aberrant mitogenic signals.

We showed that the *Ink4/Arf* locus is completely silenced in iPS cells, as well as in embryonic stem (ES) cells, acquiring the epigenetic marks of a bivalent chromatin domain, and retaining the ability to be reactivated after differentiation. Cell culture conditions during reprogramming enhance the expression of the *Ink4/Arf* locus, hence the importance of silencing the locus to allow proliferation and reprogramming. The three reprogramming factors collectively repress the *Ink4/Arf* locus soon after their expression and concomitant with the appearance of the first molecular markers of 'stemness'. The silencing of the locus is intrinsic to reprogramming and not the result of a selective process.

We found that genetic inhibition of the *Ink4/Arf* locus increased both the kinetics of reprogramming and the number of emerging iPS cell colonies (Figure 1). In

mice, *Arf*, rather than *Ink4a*, is the main barrier to reprogramming by activation of p53 and p21. In humans, however, *INK4a* is more important than ARF. Organismal ageing up-regulates the *Ink4/Arf* locus and reprogramming is less efficient in cells from old organisms; this defect can be rescued by inhibiting the locus with a short hairpin RNA. We conclude that the silencing of the *Ink4/Arf* locus

A



B

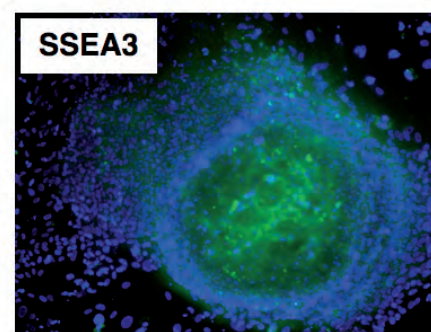
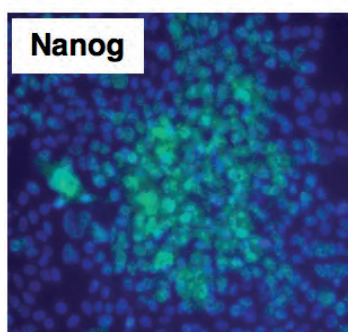


Figure 1: Impaired reprogramming of human cells by inhibition of p16^{Ink4a}. (A) Reprogramming plates treated or not with an inhibitor of p16^{Ink4a} (shINK4a). In the presence of the inhibitor the number of iPS colonies increases significantly. (B) Human iPS cells generated with shINK4a express stemness markers, such as Nanog and SSEA3 (green fluorescence).

is rate-limiting for reprogramming, and its transient inhibition may significantly improve the generation of iPS cells.

MSK2 inhibits p53 activity in the absence of stress

We had previously identified the mitogen- and stress-activated kinase 2 (MSK2) as a negative regulator of the transcription factor p53. We have now investigated the molecular mechanisms underlying the inhibition of p53 by MSK2. We found that in the absence of stress stimuli, MSK2 selectively suppresses the expression of a subset of p53 target genes. This basal inhibition of p53 by MSK2 is independent of its kinase activity and of upstream mitogen-activated protein kinase signalling to MSK2.

We have also found that MSK2 interacts with and inhibits the p53 co-activator p300, and associates with the *Noxa* promoter. We observed that apoptotic stimuli promoted the degradation of MSK2, thus relieving its inhibitory effect on p53 and enabling efficient p53-dependent transactivation of *Noxa*,

which contributed to apoptosis. Our findings constitute a new mechanism for the regulation of p53 transcriptional activity in response to stress (Figure 2).

Role of ATM in oncogene-induced senescence and p53-dependent tumour suppression

It has been recently proposed that oncogenic signalling triggers cellular senescence through the induction of DNA damage. This model has also placed *Atm* as a critical mediator of senescence in response to oncogenes. In collaboration with the CNIO Genomic Instability Group we tested this model in a variety of murine experimental systems.

We observed that over-expression of oncogenic Ras in mice fibroblasts efficiently induced senescence but this occurred in the absence of detectable DNA damage signalling, suggesting a fundamental difference between human and murine cells. We also observed that lung adenomas initiated by endogenous levels of oncogenic K-Ras presented abundant senescent cells yet undetectable DNA damage signalling. K-Ras-driven

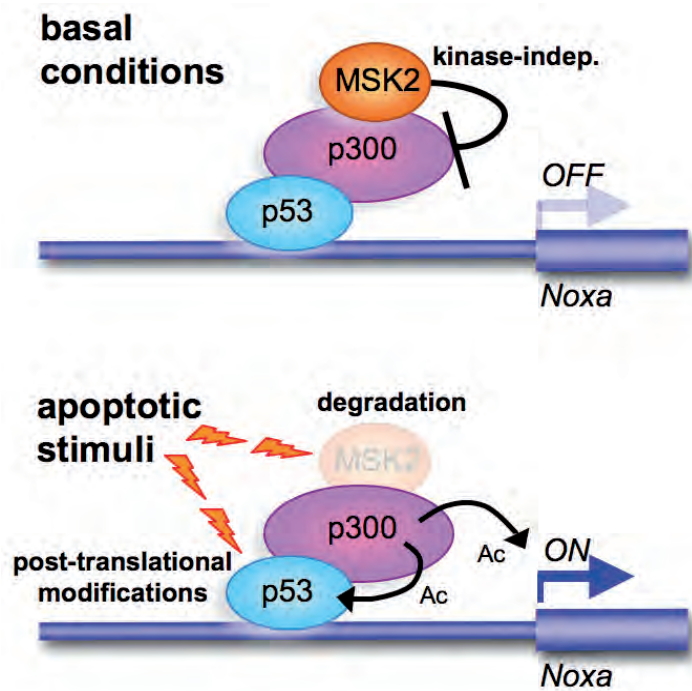


Figure 2: Model for MSK2 regulation of p53 activity at the *Noxa* promoter. In the absence of stimuli, MSK2 suppresses *Noxa* transcription by interfering with p300 cotranscriptional activity and p300-mediated p53 acetylation. Apoptotic stimuli trigger MSK2 degradation, alleviating the repression of p300 and enabling p53-mediated transcriptional activation of the *Noxa* promoter.

adenomas were also senescent in *Atm*-null mice, and the tumourigenic progression of these lesions was only slightly accelerated by *Atm*-deficiency.

We examined chemically-induced fibrosarcomas which possess a persistently activated DNA damage response and are also highly sensitive to the activity of p53. We found that the absence of *Atm* favoured genomic instability in the resulting tumours, but did not affect the persistent DNA damage response or impair p53-dependent tumour suppression.

We conclude that oncogene-induced senescence in mice may occur in the absence of a detectable DNA damage response. Our data suggest that murine

Atm plays a minor role in oncogene-induced senescence or in p53-dependent tumour suppression, and that its tumour suppressive activity is probably limited to the maintenance of genomic stability.

Publications

Li H, Collado M, Villasante A, Strati K, Ortega S, Cañamero M, Blasco MA, Serrano M (2009). The *Ink4/Arf* locus is a barrier for iPS cell reprogramming. *Nature* 460, 1136-1139.

Marión RM, Strati K, Li H, Murga M, Blanco R, Ortega S, Fernández-Capetillo O, Serrano M, Blasco MA (2009). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature* 460, 1149-1153.

Marion RM, Strati K, Li H, Tejera A, Schoeftner S, Ortega S, Serrano M, Blasco MA (2009). Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. *Cell Stem Cell* 4, 141-154.

Fernandez-Marcos PJ, Abu-Baker S, Joshi J, Galvez A, Castilla EA, Cañamero M, Collado M, Saez C, Moreno-Bueno G, Palacios J, Leitges M, Serrano M, Moscat J, Diaz-Meco MT (2009). Simultaneous inactivation of Par-4 and PTEN *in vivo* leads to synergistic NF- κ B activation and invasive prostate carcinoma. *Proc Natl Acad Sci USA* 106, 12962-12967.

Matheu A, Maraver A, Collado M, Garcia-Cao I, Cañamero M, Borras C, Flores JM, Klatt P, Viña J, Serrano M (2009). Anti-aging activity of the *Ink4/Arf* locus. *Aging Cell* 8, 152-161.

Hussain S, Romio L, Saleem M, Mathieson P, Serrano M, Moscat J, Diaz-Meco M, Scambler P, Koziell A (2009). Nephron deficiency activates NF- κ B and promotes glomerular injury. *J Am Soc Nephrol* 20, 1733-1743.

Binet R, Ythier D, Robles AI, Collado M, Larrieu D, Fonti C, Brambilla E, Brambilla C, Serrano M, Harris CC, Pedoux R (2009). WNT16B is a new marker of cellular senescence that regulates p53 activity and the phosphoinositide 3-kinase/AKT pathway. *Oncogene* 28, 9183-9191.

Lara E, Mai A, Calvanese V, Altucci L, Lopez-Nieva P, Martinez-Chantar ML, Varela-Rey M, Rotili D, Nebbioso A, Roperio S, Montoya G, Oyarzabal J, Velasco S, Serrano M, Witt M, Villar-Garea A, Imhof A, Mato JM, Esteller M, Fraga MF (2009). Salmeterol, a Sirtuin inhibitor with a strong cancer-specific proapoptotic effect. *Oncogene* 28, 781-791.

Sporn JC, Kustatscher G, Hothorn T, Collado M, Serrano M, Muley T, Schnabel P, Ladurner AG (2009). Histone macroH2A isoforms predict the risk of lung cancer recurrence. *Oncogene* 28, 3423-3428.

Artero-Castro A, Callejas FB, Castellvi J, Kondoh H, Carnero A, Fernández-Marcos PJ, Serrano M, Ramón y Cajal S, Leonart ME (2009). Cold-inducible RNA-binding protein bypasses replicative senescence in primary cells through extracellular signal-regulated kinase 1 and 2 activation. *Mol Cell Biol* 29, 1855-1868.

Trakala M, Arias CF, García MI, Moreno-Ortiz MC, Tsilingiri K, Fernández PJ, Mellado M, Díaz-Meco MT, Moscat J, Serrano M, Martínez-A C, Balomenos D (2009). Regulation of macrophage activation and septic shock susceptibility via p21(WAF1/CIP1). *Eur J Immunol* 39, 810-819.

Artero-Castro A, Kondoh H, Fernández-Marcos PJ, Serrano M, Ramón y Cajal S, Leonart ME (2009). Rpl1 bypasses replicative senescence and contributes to transformation. *Exp Cell Res* 315, 1372-1383.

Agherbi H, Gaussmann-Wenger A, Verthuy C, Chasson L, Serrano M, Djabali M (2009). Polycomb mediated epigenetic silencing and replication timing at the *INK4a/ARF* locus during senescence. *PLoS ONE* 4, e5622.

Efeyan A, Murga M, Martinez-Pastor B, Ortega-Molina A, Soria R, Collado M, Fernández-Capetillo O, Serrano M (2009). Limited role of murine ATM in oncogene-induced senescence and p53-dependent tumor suppression. *PLoS ONE* 4, e5475.

Llanos S, Cuadrado A, Serrano M (2009). MSK2 inhibits p53 activity in the absence of stress. *Sci Signal* 2, ra57.