

# Tumour Suppression Group

28

Scientific Report 2010 *crío*



Manuel Serrano

## Group Leader

Manuel Serrano obtained his PhD in 1991 for his research at the *Centro de Biología Molecular "Severo Ochoa"* under the supervision of M. Salas and J.M. Hermoso. 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. In 1997, he returned to Spain to start his own research group at the *Centro Nacional de Biotecnología*. He moved to the CNIO in 2003 to lead the Tumour Suppression Group.

His major research achievements are:

- The discovery, cloning and characterisation of tumour suppressor p16, which defined a new class of cell cycle regulators and was soon acknowledged as one of the main tumour suppressors. In addition, characterisation of p16 paved the way to another paramount tumour suppressor discovery, p19Arf, a key activator of p53.
- The establishment of the concept of "oncogene-induced senescence" as a tumour suppression mechanism. This concept rapidly became an intense research topic in many laboratories and has since been widely accepted.
- Pioneering the generation of cancer-resistant mice with the so-called "super-mice". This work demonstrated the possibility of increasing cancer resistance in the absence of deleterious secondary effects. Moreover, it revealed that tumour suppressor genes not only protect against cancer but also against ageing, in general thanks to the capacity of these genes to eliminate cellular damage.
- Characterisation of the role of p16 and p19Arf as barriers during the process of nuclear reprogramming to pluripotent stem cells.

Manuel Serrano has received numerous awards including the FEBS Anniversary Prize, the Carcinogenesis Young Investigator Award, as well as Spanish awards from *Fundación Echevarne*, *Fundación Banco de Sabadell*, and *Fundación "Carmen y Severo Ochoa"*. Manuel Serrano is an elected EMBO Member.

## Summary

Tumour suppressors are genes that can prevent the development of cancer. All our cells have a functional set of these genes. However, 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, in combination with additional mutations in other genes, may give rise to the development of cancer.

Tumours entail intrinsic aberrant conditions that normally would be highly stressful for cells. An important feature of tumour cells, however, is their capability to survive and multiply in stressed environments. One of the most common responses to stress is the permanent arrest of cell proliferation (cellular senescence). Understanding how tumour suppressor genes work may help to design drugs that block cancer growth. We manipulate the mouse genome to create novel alterations that increase or decrease tumour suppression potency.

## Strategic Goals

- To understand the mechanisms of tumour suppression and identify new tumour suppressor regulators
- Study the interplay between tumour suppression and ageing
- Characterise cellular senescence as a tumour suppression mechanism
- Study the involvement of tumour suppressors in the regulation of nuclear reprogramming of differentiated cells to induced pluripotent stem (iPS) cells
- Explore the connections between tumour suppressors and metabolism



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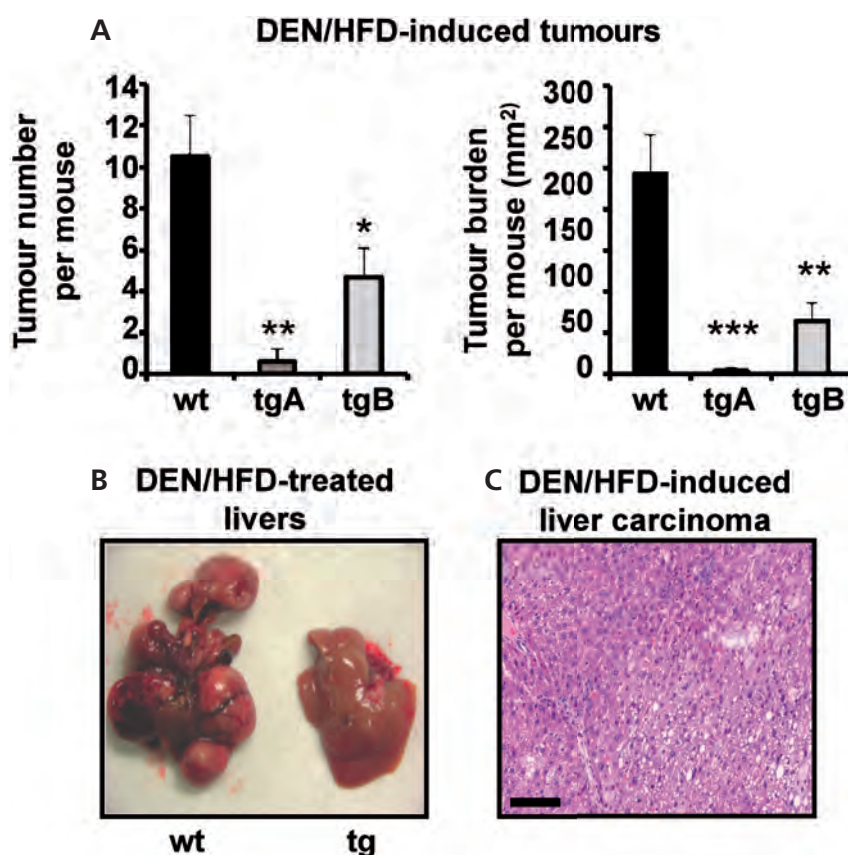
## Highlights

### Sirt1 improves healthy ageing and protects against metabolic syndrome-associated cancer

Genetic overexpression of protein deacetylase Sir2 in yeasts, flies and worms has the effect of extending longevity. Mammals have evolved a family of Sir2-related proteins known as sirtuins, composed of seven members of which Sirt1 is the closest homologue to Sir2. However, little is known to date about the impact of mammalian Sirt1 on cancer and ageing.

We have previously reported that transgenic mice systemically overexpressing Sirt1 (~3-fold) are protected from the physiological damage produced by a high-fat diet (HFD). At a molecular level, protection against a HFD reflects the activity of Sirt1 as a negative regulator of nuclear factor  $\kappa$ B (NF- $\kappa$ B) and a positive effector of PGC1 $\alpha$  and FoxO1. Chronic exposure to high levels of dietary fat results in the so-called metabolic syndrome (a multi-systemic deterioration characterised by insulin resistance, liver steatosis, atherogenic cardiovascular disease, dyslipidaemia and systemic inflammation), which may lead to liver cancer and heart failure.

We have now examined the ageing and longevity of our Sirt1-tg mice, as well as their susceptibility to spontaneous cancer and to liver cancer associated with metabolic syndrome. We observed that



**Figure 1:** Sirt1 protects from metabolic syndrome-associated liver cancer. (A) Tumour number and tumour burden in mice injected with the hepatic-specific carcinogen diethylnitrosamine (DEN) and maintained for 11 months under a high-fat diet (HFD). Tumours were measured by microCT. Tumour burden per mouse was obtained by adding the areas of the biggest transversal section of each tumour. All assays were performed with male mice ( $n \geq 5$  per group). Values are given as an average  $\pm$  s.e.m.; statistical analyses are relative to WT controls and determined by two-tailed Student's *t*-test: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.005$ . (B) Macroscopic view of representative livers from littermate mice of the indicated genotypes 11 months after initiation of the DEN/HFD protocol. (C) Histology of a representative liver carcinoma induced by the DEN/HFD protocol. Haematoxylin and eosin staining. Scale bar: 100  $\mu$ m.

old Sirt1-tg mice present lower levels of DNA damage, decreased expression of the ageing-associated gene p16Ink4a, a better health in general and fewer spontaneous carcinomas and sarcomas. These effects, however, were not sufficiently potent to affect longevity. Nonetheless, Sirt1, at least at the overexpression levels achieved in our Sirt1-tg mice, had a positive effect on health at old age, as reflected by a lower glucose intolerance, decreased osteoporosis and lower cancer incidence. Regarding liver cancer associated to obesity, Sirt1-tg mice showed dramatically increased resistance (Figure 1). Collectively our data demonstrate the tumour suppressive activity of Sirt1, which is particularly potent in the case of liver cancer associated to obesity.

### Depletion of ribosomal protein L37 induces p53

Tumour suppressor p53 is key in cancer protection due to its pivotal role in preventing damaged cells from becoming malignant. Loss of function of p53 is a frequent event in human cancers. In normal cells, p53 activity has to be tightly controlled. This regulation is accomplished primarily by the proto-oncoprotein MDM2, a ubiquitin-ligase that binds and inhibits p53 under physiological conditions. Inhibition of MDM2 function is a universal requirement for p53 activation. There are three main stress signalling pathways that lead to MDM2 inhibition: 1) DNA damage induces phosphorylation of both MDM2 and p53, reducing their mutual affinity and activating p53; 2) oncogenic signals upregulate tumour suppressor p19Arf, which binds and inhibits MDM2 thus stabilising p53; and, 3) ribosomal stress or perturbation of ribosomal biogenesis. The latter is a p53-

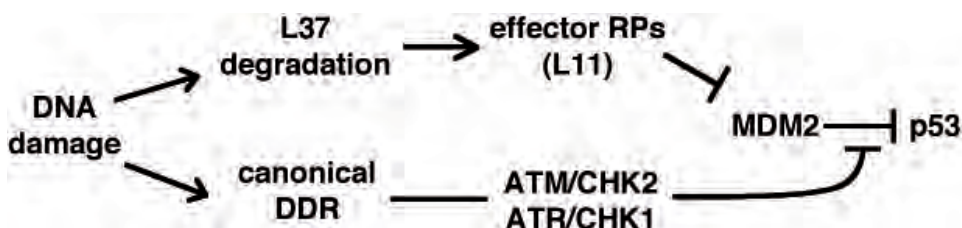
activating pathway recently discovered but gaining progressive recognition as an important regulator of p53.

Ribosomal biogenesis is perturbed by the depletion of certain ribosomal proteins (RPs), which is followed by the binding of RP L11 to MDM2, inhibition of MDM2 ubiquitin-ligase activity, and stabilisation of p53. We have observed that depletion of RP L37 leads to cell cycle arrest in an L11- and p53-dependent manner. More interestingly, we have found that treatment with UV light, cisplatin and doxorubicin (all of them genotoxic insults), leads to the degradation of L37 in the nucleoplasm and to an L11-dependent stabilisation of p53 (Figure 2). We also found that ectopic L37 overexpression can attenuate the DNA damage response mediated by p53. Our work has demonstrated that DNA damage can also be sensed by perturbations in ribosomal biogenesis, thus linking cell growth and cell division to genotoxic stress via p53.

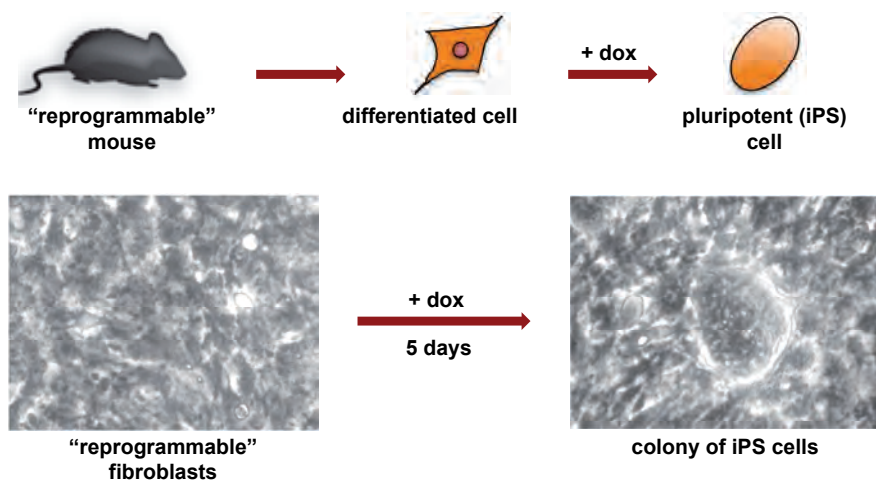
### Generation of a 'reprogrammable' mouse

The combination of four transcription factors, specifically, Oct4, Klf4, Sox2 and cMyc (abbreviated as OKSM) in conjunction with an appropriate cell culture milieu, is capable of erasing the epigenetic memory of differentiated cells converting them to a pluripotent state similar to embryonic stem cells, known as *induced Pluripotent Stem* (iPS) cells. The mechanisms underlying this process are under extensive investigation.

To facilitate the study of reprogramming, we have generated a mouse model that contains an OKSM inducible expression cassette in all its cells. This system allows



**Figure 2:** L37 and L11 levels modulate the DNA damage response. Model summarising our data. We propose that the ribosomal stress pathway contributes to the activation of p53 by DNA damage through the canonical DNA damage response (DDR) pathway. DNA damage may trigger the ribosomal stress pathway by degrading L37.



**Figure 3:** The 'reprogrammable' mouse allows the generation of induced pluripotent cells (iPS) from any differentiated cell by the simple addition of a compound known as doxycycline (dox). The lower part of the figure shows an example using fibroblasts.

reprogramming by the simple addition of doxycycline (Figure 3). A more ambitious goal is to study the consequences of a transient expression of OKSM *in vivo*. At present nothing is known about the effect of OKSM in a mammalian organism. Several scenarios are conceivable upon transient activation of OKSM: i) full reprogramming of some cells and the generation of teratomas; and ii) partial dedifferentiation of some cells which could result in either detrimental effects such as hyperplasias, or beneficial effects such as an improved regeneration potential. These different outcomes may depend on the duration of the expression of OKSM, as well as, on the particular tissue type. Exploring of these possibilities is an immediate goal of our laboratory.

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