Overexpression of wild-type *RRAS2* as a driver in breast cancer development

Claudia Cifuentes[1], Clara L. Oeste[1] , María Isabel Fernández Pisonero[2],
Xosé R. Bustelo[2], Martín C. Hörndler[3], Balbino Alarcón[1]

[1] Cell Biology and Immunology Department, Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain
[2] Cancer Research Center (CIC-CSIC), Salamanca, Spain
[3] Biobank of the Aragon Health System – Universitary Hospital Miguel Servet

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BACKGROUND

The small GTPase RRas2 is closely related to the Ras subfamily of proteins and has a similar, or even higher, transformation capacity than classical Ras proteins[1,2]. Instead of being mutated, RRAS2 has been found overexpressed in the wild-type form in human cancer cell lines and freshly isolated tumors, including oral squamous cell carcinoma[3,4], aggressive skin cancers[5], hepatocarcinoma[6]. and a variety of lymphoma subtypes[7].
The idea that the dose of RRAS2 might be relevant in human cancer is supported by the fact that RRas2 has a high intrinsic exchange rate, meaning that, in the absence of activating mutations or regulatory GEFs, a sizeable proportion of RRas2 is in the active, GTP form [8].

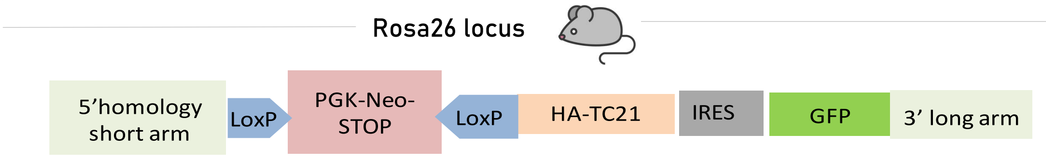
AIMS

· To explore the possible effects of wild-type RRas2 overexpression on breast cancer through the generation of mouse models.

· To characterize the downstream effects of reducing RRas2 dose in human breast cancer cell lines and ex vivo mouse tumors.

· To analyze RRAS2 expression in breast cancer patients.

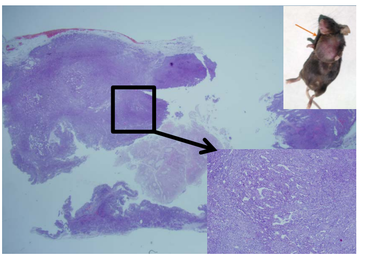
1 Generation of new mouse models



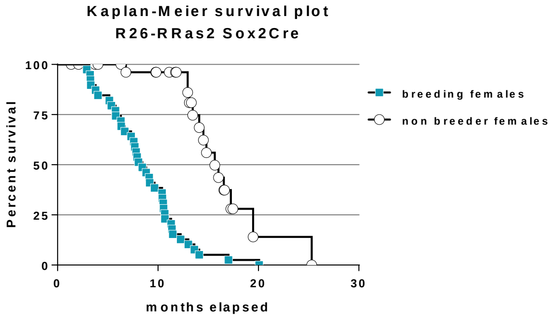
We inserted a stop sequence flanked by LoxP sites prior to wild-type HA-RRAS2. An IRES site downstream independently controls GFP expression, used as a recombination marker.

R26-RRas2 x Sox2Cre → ubiquitous

R26-RRas2 x MMTVCre
R26-RRas2 x WapCre → tissue-specific → Epithelial cells, Secretory epithelial cells



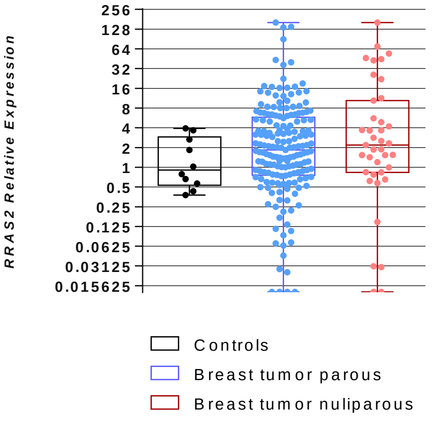
Detection of breast cancer in females of the Sox2-Cre x R26-Rras2fl/ fl mouse line.
A tumor mass developed in the upper ventral part of a 4 month-old female that have had two litters. The mass was determined after hematoxylin/eosin staining and pathologist's evaluation to be a ductal breast carcinoma.



All mice developing breast tumors were mating females, with a mean onset after first mating of 7 months.

A high penetrance of this type of tumor has been observed in females. The earliest age of tumor onset is 14 weeks, with a mean of 33,7 weeks.
Female mice that had one or more litters showed a decreased survival rate, compared to non breeders. Non breeder females died of old age or developed other type of tumors, like cholangiocarcinomas.

2 68% of breast cancer patients overexpress *RRAS2*



RTqPCR analysis developed in 284 breast cancer samples showed that **68% of patients expressed *RRAS2* at higher dose than normal breast tissue samples. 48% expressed more than double.**

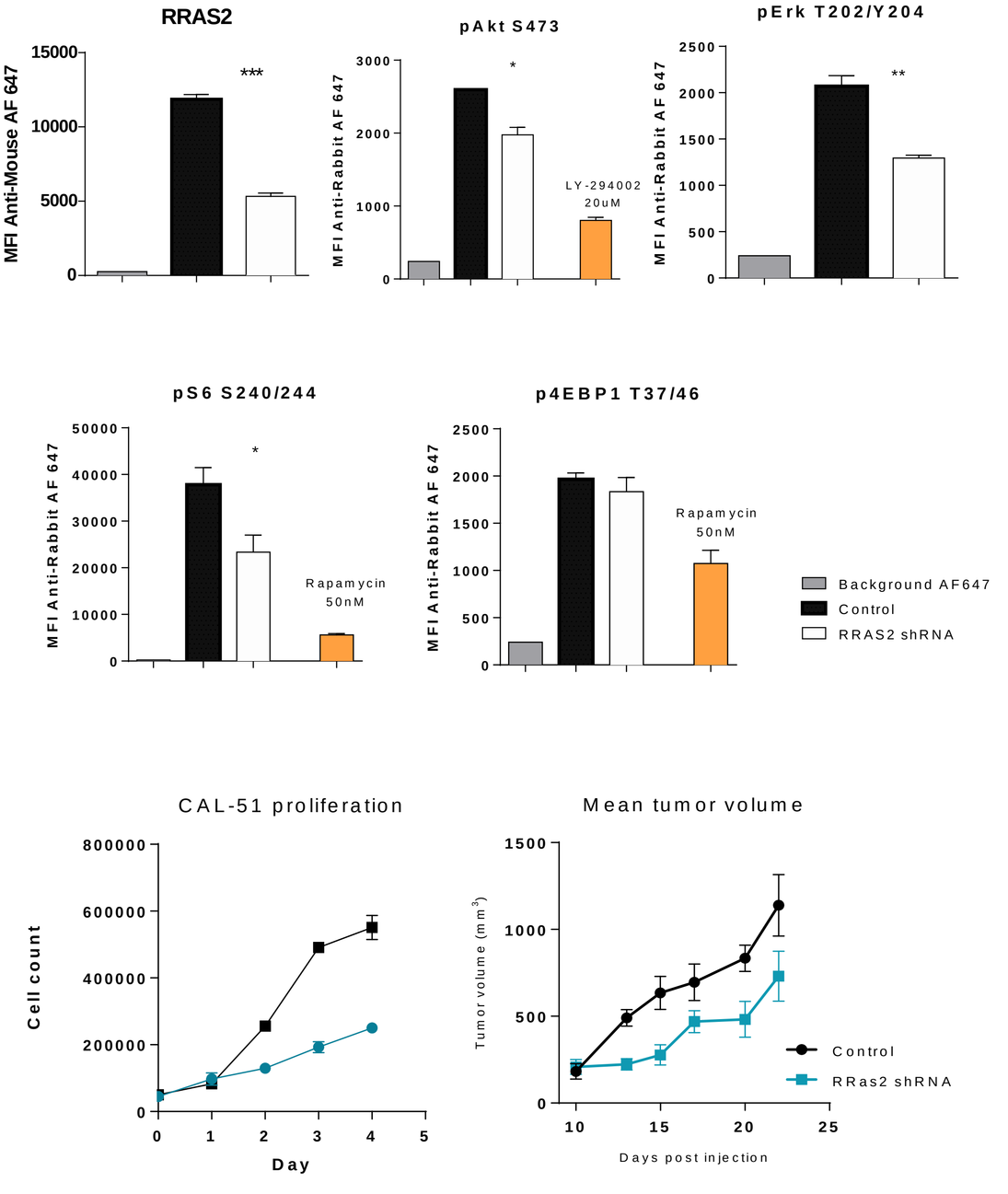
In addition, women between 40-55 years old seemed to express significantly higher levels than other groups of age.

3 RRas2 downregulation decreases PI3K/mTor and MAPK signaling pathways

We generated stable transductants with **RRAS2 shRNA lentiviral particles** using:

· CAL-51 cell line. Human triple negative breast cancer cell line that overexpresses oncogenic *RRAS2* with Q72L mutation (constitutively active).

· *Ex vivo* cell line generated from a R26-RRas2 x Sox2Cre breast tumor.



Reduction of RRas2 levels showed a decrease in phosphorylation in the MAPK and PI3K/mTor pathways. This reduction leads to depletion of proliferative activity *in vitro* and *in vivo*.

Conclusions

RRas2 levels are important for mammary tissue homeostasis and plays a role in tumorigenic processes.

·Each of our three non-mutated RRas2-overexpressing mouse lines develop breast carcinomas in breeder females .

·Non-breeders females don't show any type of breast tumor.

·The PI3K/mTor and MAPK signaling pathways are affected by RRas2 dose. Phosphorylation levels decrease, having effects on tumorigenic activity.

Bibliography

1 - Drivas, G.T et al. (1990). Mol and Cell Biol. 10: 1793-1798.
2- Graham, S.M. et al. (1994). Mol and Cell Biol 14: 4108-4115.
3- Arora, S. et al. (2005). Mol Carcinog 42: 97-108.
4- Macha, M.A. et al. (2009). J Oral Pathol Med 39: 477-485.
5- Lee, J.H. et al. (2011). Int J Dermatol 50: 956-960
6- Luo, H. et al. (2010). Int J Oncol 37: 853-859.
7- Delgado, P. et al. (2009). Nat immunol 10: 880-888.
8- Larive, R.M. et al. (2014). Nat Commun. 5: 3881