Understanding the relevance of a new biomarker in the development and prognosis of most common cancers

Validación de un nuevo biomarcador del desarrollo y prognosis de los cánceres más frecuentes

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Top 10 First or Corresponding Author Publications


TOP 5 FUNDING GRANTS

- 2022-2023. Modelo en ratón de leucemia linfócita crónica (LLC) para la validación de nuevas intervenciones terapéuticas. PDC2021-121170-I00. 150.000 €
- 2018-2019. Desarrollo preclínico de nuevos fármacos para el tratamiento personalizado de cánceres dependientes de Ras. RTC-2017-6478-1. Allinky Biopharma y CSIC. 120.000 € (BA)
- 2016-2021. Función, valor diagnóstico e inhibición farmacológica de RRas2, un nuevo “driver” oncogénico. Fundación Científica de la Asociación Española Contra el Cáncer. Grupos Estables (Bustelo, Caballero, Oaknin y Alarcón). 400.000 € (BA).

TOP 3 TRANSFER ACTIVITIES


SECTIONS TO COMPLETE – SCIENTIFIC PROPOSAL (MAX. 3 PAGES)

BACKGROUND AND CURRENT STATUS OF THE TOPIC (MAX. ½ PAGE)

R-RAS2 (also known as TC21) belongs to the R-RAS subfamily and is the RAS superfamily member that shows the closest structural similarity with classical RAS GTPases ¹, and the only GTPase that displayed transforming activities similar to, or even higher than those exhibited by H-, N-, and K-RAS proteins²-⁴. This observation led to the idea that R-RAS2 could be a surrogate protumorigenic route in tumors that lacked oncogenic mutations in classical RAS genes. However, different studies indicated that the RRAS2 gene did not seem to be significantly mutated in human cancer. This idea prevailed in spite the fact that amino acid Q72 in R-RAS2 has been identified as a mutational hotspot in human cancer⁵. Indeed, we have recently shown that expression of the Q72L mutant in mice causes the development of T cell and B cell acute leukemias⁶. Notwithstanding, there is still a wide gap between the overall mutagenesis rate in KRAS and RRAS2 genes in human cancer (14% vs. 0.8%) and unlike for KRAS, most of the genomic changes involve gene amplification and not missense mutations⁷. Same pattern holds true for all types of human breast cancer (BC). RRAS2 has been found overexpressed in the wild type form at the mRNA and/or protein levels in human cancer, including BC ⁸. In spite the frequent finding of RRAS2 mRNA and/or protein overexpression in human cancer, a causal relationship had not been established. In order to study if RRAS2 overexpression causes cancer, we have generated a conditional overexpression knock-in mouse line with a RRAS2 construct in the Rosa26 locus to create a line (R26-RRAS2) bearing the recombined locus in the germline. In this line, we detected the emergence of chronic lymphocytic leukemia (CLL) in all mice, male and female⁹, and breast ductal adenocarcinomas in female mice but just in mating females (Cifuentes et al, submitted). Therefore, the association of RRAS2 overexpression with BC could be especially relevant for those cancers associated to childbearing. In addition, the involvement of RRAS2 in BC goes along with our previous data indicated that Ras2/RRAS2 is involved both in the physiological development of the mammary gland at puberty and in the proliferative and metastatic potential of BC cell lines⁹,¹⁰. Further links between RRAS2 expression and BC were found by others showing that a polymorphism in the promoter region (-582C>T) conducted to higher R-RAS2 protein
expression and increased likelihood of relapse in response to tamoxifen monotherapy. In addition, in a functional screen in an ER+ human BC cell line, RRAS2 was identified as one of the few genes whose expression confers resistance to tamoxifen treatment. The frequent overexpression of RRAS2 mRNA in CLL (80%) and breast cancer (68%) suggests that RRAS2 overexpression is behind the development of those cancers. However, perhaps the most compelling support for a genetic linkage between RRAS2 and cancer is the finding of SNP rs8570 in the 3'-UTR of the RRAS2 mRNA which is not found at equilibrium in CLL and BC. This SNP was originally identified as one affecting genes in the MAPK pathway that are associated to cutaneous melanoma. We have recently demonstrated that overexpression of wild type RRAS2 drives the development of both CLL and breast cancer in mice and that this correlates with a frequent overexpression of the gene in those two types of human cancer. The non-random distribution of the SNP rs8570 G and C alleles in CLL, but especially in BC, and its association with RRAS2 overexpression, suggest that RRAS2 is behind the development of the majority of those two types of cancer. A contingency test of the homozygote distribution (GG vs CC) in 378 BC patients versus healthy volunteer cohorts showed a very strong disequilibrium in the number of GG homozygotes in favor of CC homozygotes within the tumor samples. This SNP was originally identified as one affecting genes in the MAPK pathway that are associated to cutaneous melanoma. We have recently demonstrated that overexpression of wild type RRAS2 drives the development of both CLL and breast cancer in mice and that this correlates with a frequent overexpression of the gene in those two types of human cancer. The non-random distribution of the SNP rs8570 G and C alleles in CLL, but especially in BC, and its association with RRAS2 overexpression, suggest that RRAS2 is behind the development of the majority of those two types of cancer. A contingency test of the homozygote distribution (GG vs CC) in 378 BC patients versus healthy volunteer cohorts showed a very strong disequilibrium in the number of GG homozygotes in favor of CC homozygotes within the tumor samples. These findings place SNP rs8570 as an important biomarker for the identification of the risk of developing BC and its prognosis.

HYPOTHESIS AND OBJECTIVES (MAX. ½ PAGE)

In this project we aim to investigate by means of measuring the SNP allele frequencies if RRAS2 is also likely to be involved in other human cancers. In addition, we propose to investigate if there are relationships and prognosis and survival in CLL, breast cancer and other cancers. We also aim to characterize possible microRNAs that differentially regulate the stability of the RRAS2 mRNA bearing the G and C alleles and the functional interaction between R-RAS2 protein and membrane proteins involved in breast cancer survival, proliferation, metastasis and stemcellness. In summary, with this project we aim to validate the relevance of SNP rs8570 as a predictive biomarker and of RRAS2 as a new cancer driver.

**Objective 1.** To determine the frequency of G and C alleles at SNP rs8570 in different types of human cancer.

**Objective 2.** To investigate the relationship between expression of the G and C alleles of the SNP rs8570 and prognosis in human cancer.

**Objective 3.** To characterize possible microRNAs that differentially regulate the stability of RRAS2 mRNA at the SNP position.

**Objective 4.** To investigate the relevance of R-RAS2 interaction with key membrane proteins in breast cancer cells for proliferation, survival metastasis and stemcellness phenotype.

METHODOLOGY (MAX. 1 PAGE)

**Objective 1.** This Objective will involve the use of qPCR method to distinguish the G and C alleles of the SNP already developed in the laboratory and the acquisition of genomic DNA samples from Spanish Biobanks. The Biobank of the Aragón Health System and the CNIO have already been contacted and will be providing high-quality samples. 1.1) Assessment of the frequency of the two SNP alleles in T and B cell lymphomas and leukemias. 1.2) Assessment of the frequency of the two SNP alleles in carcinomas described to overexpress RRAS2: lung non-small cell squamous carcinomas and adenocarcinomas, billiary duct adenocarcinomas, liver hepatocellular carcinoma, ovary adenocarcinoma and cervix squamous carcinoma.

**Objective 2.** 2.1) Association between GG, GC and CC genotypes at SNP site with necessity for targeted therapies, drug resistance and overall survival in CLL. 2.2) Association between SNP genotypes and response to therapy and and overall survival in breast cancer. 2.3) Association between SNP genotypes te with necessity for chemotherapy, drug
resistance and overall survival in most common cancers.

**Objective 3.** This Objective will involve the purification and sequencing of microRNAs that differentially bind the 3'-UTR of RRAS2 bearing the G vs the C alleles. Demonstration of the regulatory effect of the candidate microRNAs will be undertaken by overexpression and knockdown approaches in human BC cell lines. 3.1) Unbiased identification of microRNAs that differentially bind the 3'-UTR of RRAS2 mRNA bearing the G allele vs the C allele. 3.2) Demonstration of the regulatory effect of selected microRNAs on RRAS2 expression in cancer. 3.3) Study of the expression of microRNAs regulating RRAS2 expression in different types of cancer.

**Objective 4.** This Objective will involve the use of a murine BC cell line established in the lab from a primary tumor emerging in a female of the Rosa26-RRAS2flox/floxWap-Cre BC-prone line and knockout/knockdown approaches to determine the effect of RRAS2 depletion on the function of different membrane receptors found to interact with R-RAS2 protein. 4.1) Role of R-RAS2 on CD44-promoted breast cancer cell migration and metastasis. 4.2) Role of R-RAS2 on canonical Wnt/β-catenin signaling in breast cancer. 4.3) Role of R-RAS2 on Epha2 signaling in breast cancer. 4.4) Role of R-RAS2 on breast cancer cell stemness.

**BIBLIOGRAPHY (MAX. ½ PAGE)**


**SOCIAL AND SCIENTIFIC IMPACT OF THE PROPOSAL (MAX. ½ PAGE)**

Although men can develop breast cancer, this disease is essentially a women’s disease in fact, it is the most frequent form of cancer in women around the world (a 25.1 % of all cancers, GLOBOCAN 2012) with an estimate death toll number of more than 500,000 women per year. Therefore, this proposal focuses on an important disease for women.
A long-standing paradigm in the oncology field is that cancer is driven by activating mutations in genes such as KRAS, this proposal aims however to confirm our already solid preliminary data showing that overexpression of the WT form of RRAS2 drives cancer. This is relevant because the search for genetic modifications that conduct to malignant transformation has been mostly based on alterations that affect coding sequences. The fact that overexpression of the WT form of RRAS2 leads to breast cancer points out towards mutations in untranslated and regulatory sequences of RRAS2 as possible causes of cancer. The strong bias towards GC heterozygosis and CC homozygosis versus GG homozygosis at the position of the rs8570 SNP, strongly argues in favor or a role for RRAS2 in the majority of breast cancers. This project will serve to confirm such finding by increasing the number of analyzed patients and finding mechanistic explanations for the predominance of the GC and CC genotypes among breast cancer patients.

Nonetheless, the most important potential implications of the proposal that fits with a societal expectation is that we are going to develop both a diagnostics tool and a new therapy for breast cancer. We aim to set-up a PCR-based test to easily determine the genotype at the SNP rs8570 position. This test will serve: 1) as a prognostic tool to evaluate in the healthy population the degree of protection against breast cancer development (GG genotype); 2) in combination with a measurement by qPCR of RRAS2 mRNA expression, the likelihood that the breast cancer is driven by overexpression of wild type RRAS2, and therefore it is amenable to the new targeted therapy. But perhaps, the most important implication is that if the results in preclinical models show that AIK-324 reduces the growth or even reverts mouse and human breast tumors, the drug will be directed to clinical trials on breast cancer patients in a near future.

In summary, from a point of view of scientific knowledge, we will establish that wild type and not mutant forms of RRAS2 are oncogenic in a very important human cancer. From a translational aspect, we will develop a diagnostics tool and a therapy. From a clinical point of view, we will impulse the development of a new therapy. From a socioeconomic point of view, we will identify first the need (breast cancer patients with overexpressed RRAS2) and then offer the cure (the targeted therapy).