Data input form: MouseAGE Database

Author: Angelica Figueroa Conde-Valvís

Institution: Instituto de Investigación Biomédica A Coruña (INIBIC)

Country: Spain

Date submitted: 20/04/2018

Preferred acknowledgement:

Summarise supporting data attached:

At early stages of carcinoma progression, epithelial cells undergo a program named epithelial-to-mesenchymal transition characterized by the loss of the major component of the adherens junctions, E-cadherin, which in consequence causes the disruption of cell-cell contacts. Hakai is an E3 ubiquitin-ligase that binds to E-cadherin in a phosphorylated-dependent manner and induces its degradation; thus modulating cell adhesions. We have recently used analyzed, in vitro and in vivo, the effect of Hakai overexpression in a non-transformed Madin-Darby Canine Kidney (MDCK) cells, an established model system to study cell-cell adhesions and EMT. By using this model system we have reported that Hakai induces tumour progression and metastasis *in vitro* and *in vivo* (Castosa et al, *Sci Reports*, 2018). Interestingly, histological analysis showed a teratoma formation while injecting 5×10^6 MDCK cells subcutaneously into the flank of nude mice. In contrast, tumors induced by Hakai-MDCK cells showed a significant change to undifferentiated and spindle-shape carcinoma cells. In the present new results showed here, it was evaluated the degree of angiogensesis, by analysing CD31 immunohistochemical staining and quantification. Interestingly, it was showed found that the degree of angiogenesis in teratoma formed by injecting MDCK showed was higher compared to tumours originated by Hakai overexpressing cells.

Ageing / disease: Cancer

Model: xenograft assay with 6 week-old athymic nu/nu mice

Clinically relevant? (Yes or No): Further studies need to be performed.

Explanation of why clinically relevant or not:

Angiogenesis is an important process in cancer, indeed some angiogenesis inhibitors are used in clinical practice for cancer treatment. The fact that angiogenesis formation is higher in the teratoma induced by non-transformed MDCK compared to Hakai-ovexpressing MDCK xenografts might open the possibility of the benefits of this treatment in benign or malignant teratomas.

Characteristics, timing of appearance of phenotype and phenotypic tests used (attach supporting data when unpublished if available):
We injected $5x10^6$ MDCK or Hakai-MDCK cells into the flank of nude mice. Animals were sacrificed 38 days after injection and tissues and organs were collected, fixed in PFA and embedded in paraffin blocks for histology and/or immunohistochemistry (IHC) analyses. Angiogenesis was analysed by IHQ using CD31 antibody, an angiogenesis marker, and the number of new blood vessels in each field was quantified (Figure 1).

If applicable, Intervention(s) performed (type, dose, route of administration, frequency): Subcutaneously injection of $5x10^6$ MDCK cells or Hakai-overexpressing MDCK cells into the flank of nu/nu mice. Thirty-weight days after injection, animals were euthanized and samples were collected, fixed in 4%PFA and embedded in paraffin blocks.

Phenotype post-treatment (include null or negative results and attach supporting data separately):

Teratomas were detected in animals injected with non-transformed MDCK cells whereas aggressive and mesenchymal tumours were found in all animals injected with Hakai-MDCK cells. By IHQ with CD31 antibody, an angiogenesis marker, we observed a strong increase in the number of new blood vessels in teratoma compared to tumours. These results suggest the role of angiogenesis in teratoma formation.

References (list below):

1.- Kobayashi H and Lin PC. Angiogenesis links chronic inflammation with cancer. Methods Mol Biol. 2009;511:185-91. doi: 10.1007/978-1-59745-447-6_8.

2.- Brabletz T, Kalluri R, Nieto MA and Weinberg RA. EMT in cancer. Nat Rev Cancer. 2018 Feb;18(2):128-134. doi: 10.1038/nrc.2017.118. Epub 2018 Jan 12.

3.- Castosa R, Martinez-Iglesias O, Roca-Lema D, Casas-Pais A, Díaz-Díaz A, Iglesias P, Santamarina I, Graña B, Calvo L, Valladares-Ayerbes M, Concha Á and Figueroa A. Hakai overexpression effectively induces tumour progression and metastasis in vivo. Sci Rep. 2018 Feb 22;8(1):3466. doi: 10.1038/s41598-018-21808-w.

4.- Zinger A, C Cho W and Ben-Yehuda A. Cancer and Aging - the Inflammatory Connection. Aging Dis. 2017 Oct; 8(5): 611–627.