The Role Of JDP2 And ATF3 Expression In Angiogenesis

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1. *In-Vivo* Tumor Growth And Angiogenesis

We tracked and analyzed Lewis Lung Carcinoma (LLC) tumors in mice models, in order to find the connection between the genotypes of the host mouse and the tumor volume as well as the microenvironment. All mice were injected with identical LLC cells, as the only difference was the mouse genotype.

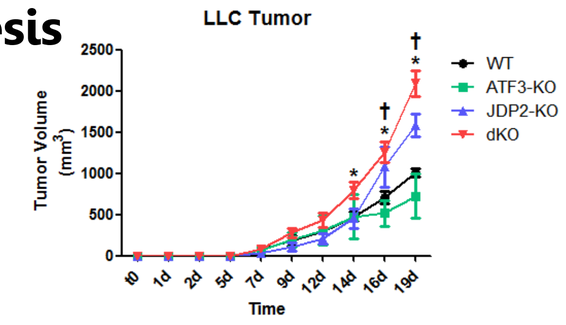

Figure 1
Our results show that JDP2-KO mice have bigger tumors than WT mice. In addition ATF3-KO show no difference and the dKO mice have a significantly bigger tumors than WT. This is a phenomena of synergism between the two transcription factors.

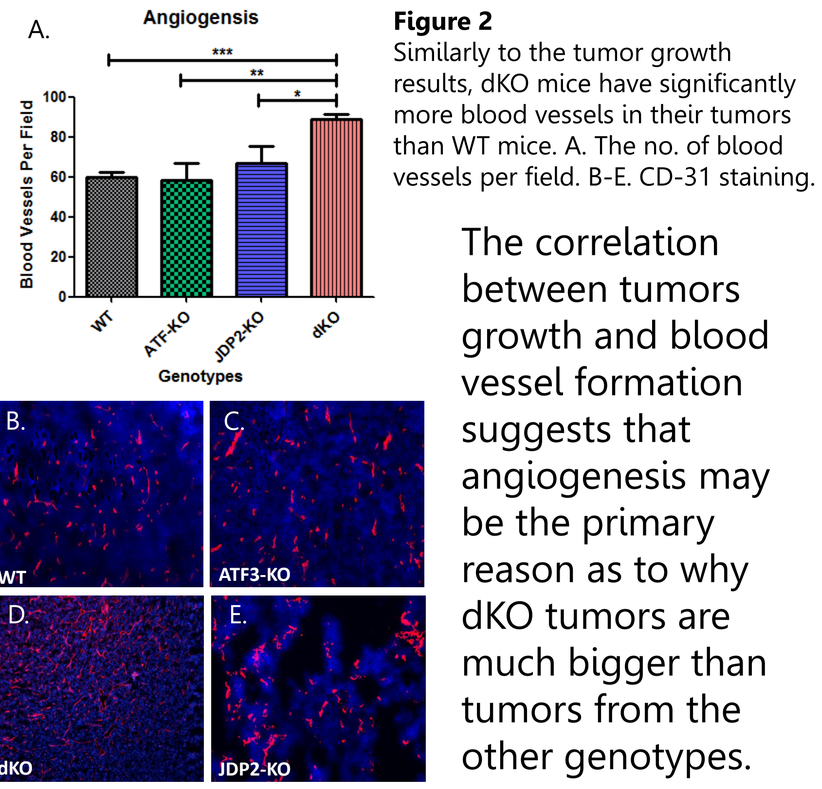

Figure 2
Similarly to the tumor growth results, dKO mice have significantly more blood vessels in their tumors than WT mice. A. The no. of blood vessels per field. B-E. CD-31 staining.

The correlation between tumors growth and blood vessel formation suggests that angiogenesis may be the primary reason as to why dKO tumors are much bigger than tumors from the other genotypes.

2. Matrigel Plugs

Matrigel is a gelatinous substance that simulates the extracellular matrix. By injecting this substance mixed with different serums into wild type mice, our goal was to observe how the serum from the different genotypes affected the recruitment of cells.

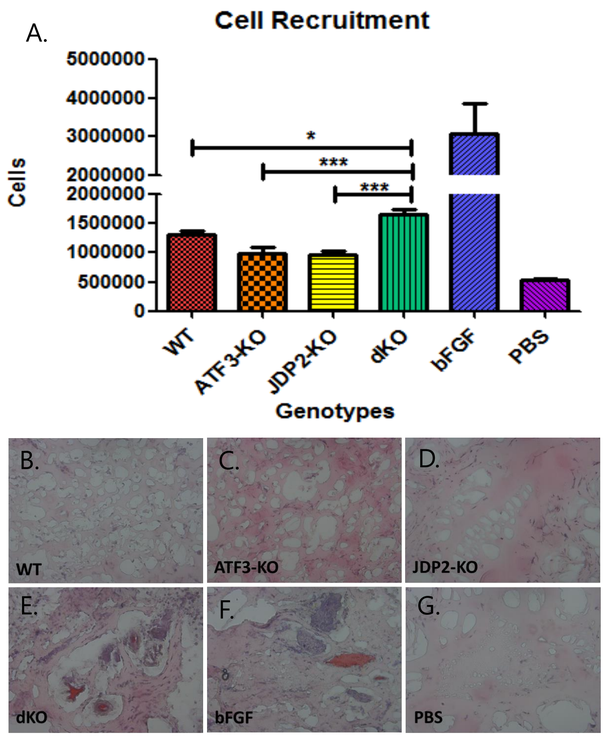

Figure 3
Our results in figure A showed that the most cells were recruited in the dKO mice, therefore suggesting that cell recruitment is one of the main components of the bigger tumor phenomena. B-G. H&E staining.

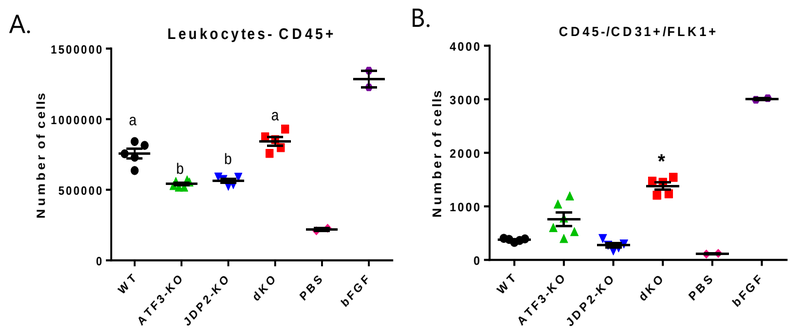

Figure 4
These graphs show the results of the FACS analysis. The CD45 staining (A) shows no significant difference in immune cell recruitment, yet the CD31 staining (B) shows a significant difference in the endothelial cell recruitment to dKO mice.

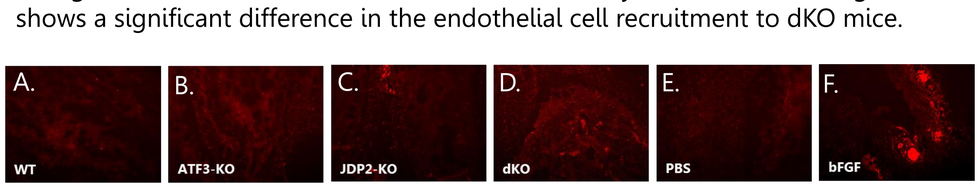

Figure 5
The immunohistochemistry staining for CD31 (A-F) shows new blood vessels formation only as a result of adding JDP2-KO and dKO serum.

Introduction

Tumors are complex tissues composed of cancer cells that interact with non-malignant cells in their surroundings known as the '**stroma**' [1].

Endothelial cells, specifically, generate cancer vasculature in a process called **angiogenesis**, which supplies growth-promoting factors and oxygen to the cancer tissue [2] The c-Jun dimerization protein 2 (**JDP2**) and Activating transcription factor 3 (**ATF3**) are transcription factors that were found to have a **dual role in malignant transformation** [3-4].

<u>In this project, we wanted to understand how the expression of ATF3 and JDP2 in host stroma affects tumor growth in relation to angiogenesis.</u>

3. Tube Formation

We seeded HUVEC cells in Matrigel and added serum of the different genotypes and recorded their tube formation.

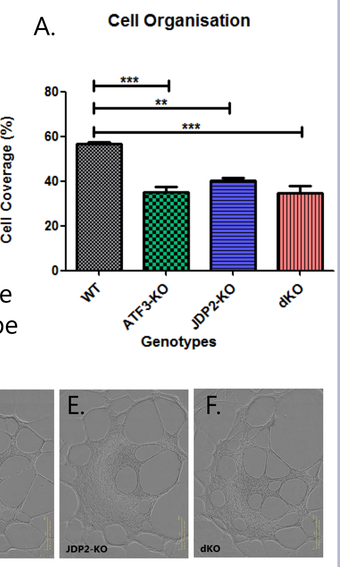

Figure 6
The higher the cell coverage, the lower the tube formation is, thus, cells in the dKO and ATF3-KO serums show higher tube formation that the cells in the WT serum. A. The percentage of cell coverage. B-F. Tube formation.

4. Cell Proliferation

Our goal was to understand if the factors within serums from different genotypes affect endothelial and cancerous cells proliferation.

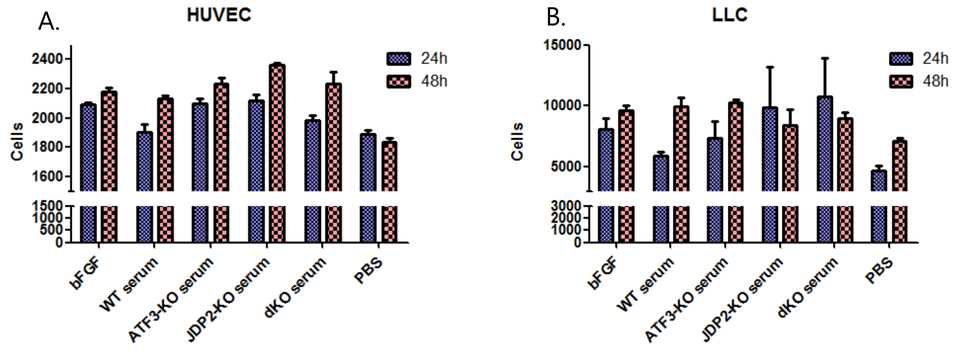

Figure 7
A. HUVEC and B. LLC cell proliferation in the presence of serum from different genotypes.

We found that the different serums **do not lead to a significant difference** in the proliferation of both HUVEC and LLC cells.

Conclusions

We found a direct link between angiogenesis and the phenomena of larger tumors in the dKO hosts, both *in-vivo* and *in-vitro*. Therefore we conclude that an increase in angiogenesis may be crucial for the formation of bigger tumors and is triggered by the knockout of ATF3 and JDP2.

Ethics

It was necessary to use live animals (mice) in our work, as both *in-vitro* and *in-vivo* experiments are needed to fully simulate the human model and give accurate results. We treated our mice with the upmost care and we checked the progression of their health daily.

Acknowledgements

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[1] D. Hanahan and R. a. Weinberg, "Hallmarks of cancer: The next generation," Cell, vol. 144, no. 5, pp. 646–674, 2011.
[2] C. B. Annika Armulik, Alexandra Abramsson, "Endothelial / Pericyte Interactions," Circulation research, vol. 97. pp. 512–523, 2005.
[3] C. C. Wolford, et al., "Transcription factor ATF3 links host adaptive response to breast cancer metastasis," J. Clin. Invest., vol. 123, no. 7, pp. 2893–2906, 2013.
[4] Y. Barbarov , et al., Host JDP2 expression in the bone marrow contributes to metastatic spread. Oncotarget, 2015.