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# Lysyl oxidase-like 3 involvement in melanoma progression

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## INTRODUCTION

Lysyl oxidase-like 3 (LOXL3) is a member of the lysyl oxidase family comprising multifunctional enzymes with depicted roles in extracellular matrix maturation, epithelial to mesenchymal transition (EMT), development, differentiation and angiogenesis, as well as in distinct pathologies such as fibrosis and cancer [1-4].

Extensive work by our lab and others has established the deregulation of lysyl oxidases in cancer and their status has been associated with patient outcome in specific neoplasias [1,2]. Our previous studies identified human LOXL3 as a modulator of EMT and Snail1 functional activity [5] and we have recently unveiled an unexpected contribution of LOXL3 to melanoma pathogenesis and described LOXL3 role in human melanoma biology [6].

Cutaneous melanoma is one of the most aggressive forms of skin cancer with a high mortality rate due to its metastatic ability. Malignant melanoma is characterized by its heterogeneity, aggressiveness and resistance to treatment. Most cutaneous melanomas on intermittently sun-exposed skin harbor mutations that constitutively activate the MAP Kinase/ERK signaling pathway, which regulates cell proliferation and survival [7]. BRAF is the most commonly mutated gene and it is already present in benign nevi indicating that further genetic alterations are required for malignant transformation [8].

## OBJECTIVE

Evaluate the *in vivo* contribution of Loxl3 to melanoma biology.

## METHODOLOGY

A combination of *in vitro* and *in vivo* approaches involving loss of function of Loxl3 in mouse melanoma B16F10 cell line have been used (proliferation, cell survival and tumorigenesis & metastasis assays). Besides, we have generated a genetically engineered mouse model with conditional deletion of *Loxl3*. This inducible Loxl3 knock-out mouse was crossed to an established melanoma genetic model that recapitulates the biology of melanoma initiation, progression and metastasis mimicking the human disease [9].

## RESULTS

We have analyzed the consequences of Loxl3 depletion in the metastatic mouse melanoma cell line (B16F10) to find that Loxl3 silencing affects negatively in vitro cell proliferation as well as anchorage-independent growth. Experimental metastasis assays by tail-vein injection in syngeneic mice of control and Loxl3-silenced B16F10 cells have also been performed observing reduced lung metastatic colonization in the absence of Loxl3 of B16 F10 cells compared to control cells.

In our conditional mice, upon 4-hydroxytamoxifen (4-HT) treatment, the Tyr::CreERT2 transgene allows melanocyte specific Cre expression promoting constitutive active mutant $BRAF^{V600E}$ expression and PTEN loss and concomitant Loxl3 deletion. Topical application of 4-HT results in the development of pigmented skin lesions which progress to malignant melanoma whereas metastasis is detected in lymph nodes and lungs. Ongoing experiments show that, in the absence of Loxl3, the onset of nevi is delayed while overall survival of mice is increased compared to control animals. Moreover, distal lymph node colonization and lung metastatic foci decrease in the absence of Loxl3 confirming that Loxl3 plays an important role in melanoma pathogenesis *in vivo*. Currently, we are deriving mouse melanoma cell lines from control and Loxl3 KO melanoma tumors from the mice in order to further evaluate the molecular mechanism underlying Loxl3 role in melanoma initiation and progression *in vivo*.

CONCLUSIONS

Loxl3 knock-down *in vivo* delays melanoma onset and progression as well as metastatic colonization confirming its key involvement in melanomagenesis.

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