TBX3 represses *CerS1* to inhibit apoptosis and promote drug resistance inmalignant melanoma

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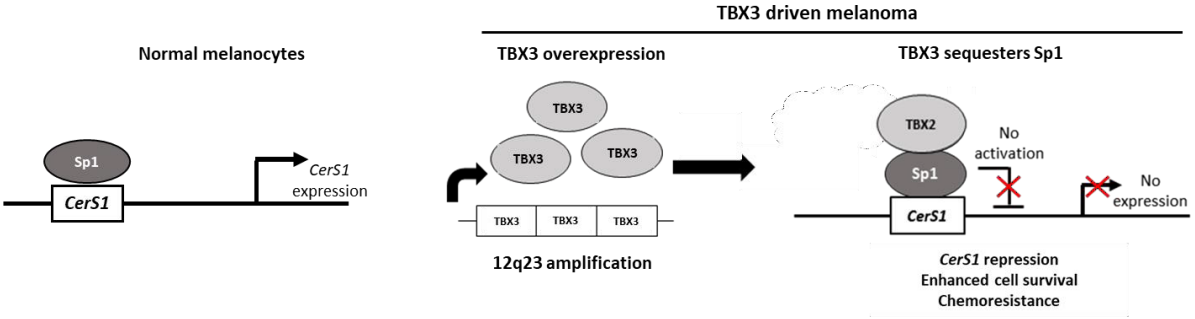

Figure: Proposed model of *CerS1* repression by TBX3 through sequestration of Sp1.

Introduction

Malignant melanoma accounts for approximately 75% of all skin cancer-related deaths due to high rates ofrecurrence and metastasis, limiting the efficacy of current therapeutic strategies (Roesch, 2015). Improvingour knowledge of the key molecular drivers of malignant melanoma will facilitate the identification of noveltargets for the development of improved therapies for the treatment of this highly intractable disease. TheT-box transcription factor, TBX3, is a key driver of melanomagenesis, where it promotes proliferation, tumour invasiveness and metastasis (Hoek et al., 2004; Rodriguez et al., 2008; Peres et al., 2010). This studyshows that TBX3 also promotes apoptosis bypass and melanoma drug resistance by repressing *ceramide synthase 1 (CerS1)*, a key enzyme involved in the synthesis of C18-ceramide, a bioactive sphingolipid shownto promote cell death and sensitize cancer cells to chemotherapeutics.

Methodology

TBX3 and CerS1 mRNA and protein levels were determined in a panel of melanoma cell lines using qRT-PCRand western blotting, respectively. Chromatin immunoprecipitation and luciferase assays were performedto test whether TBX3 directly binds and represses the *CerS1* promoter and to determine which regions of the TBX3 protein and *CerS1* promoter is involved in this repression. Lastly, western blot analyses were performed to determine the effects of TBX3 overexpression on cell survival and drug resistance to the pro-apoptotic binuclear palladacycle, AJ-5.

Results

Here we show that (1) TBX3 and CerS1 mRNA and protein levels correlated inversely in a panel of melanomacell lines; (2) TBX3 represses the *CerS1* promoter independent of a consensus T-element, through a mechanism involving its ability to directly interact with and inhibit Sp1, a reported activator of *CerS1*; (3) TBX3 does not require the DNA binding and N-terminal repression domains to repress *CerS1*; (4) the binuclear palladacycle, AJ-5, promotes apoptosis in a TBX3-CerS1 dependent manner and TBX3 overexpression confers cell survival and AJ-5 resistance.

Conclusion

This study uncovers a novel mechanism by which TBX3 represses *CerS1* by hijacking Sp1 to inhibit C18ceramide synthesis and thereby inhibit apoptosis and promote apoptotic drug resistance.

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