Medulloblastoma Oncogene OTX2 Promotes Growth in Other Tumors Via Regulation of MYC, CRX, and Phosphorylation of RB

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Introduction

The homeobox transcription factor OTX2 plays a critical role in very early neurogenesis, but can become oncogenic when aberrantly expressed later in life.

Previous work has identified OTX2 as an oncogene in medulloblastoma; however, little is known about the role of OTX2 in retinoblastoma, another childhood malignant tumor.

Methods

1. *OTX2* amplification and expression levels were assessed in primary retinoblastoma tumors and retinoblastoma cell lines Y79 and WERI
2. *In vitro* study: *OTX2* expression was disrupted in retinoblastoma cell lines via *OTX2* -specific siRNAs and all-*trans* retinoic acid (ATRA); cell apoptosis and proliferation was measured after knockdown
3. *In vivo* study: *OTX2* expression was disrupted in retinoblastoma mice xenografts via shRNAs; tumor size and tumor growth was recorded after knockdown
4. MYC, CRX, and phosphorylated RB expression levels after *OTX2* knockdown was measured

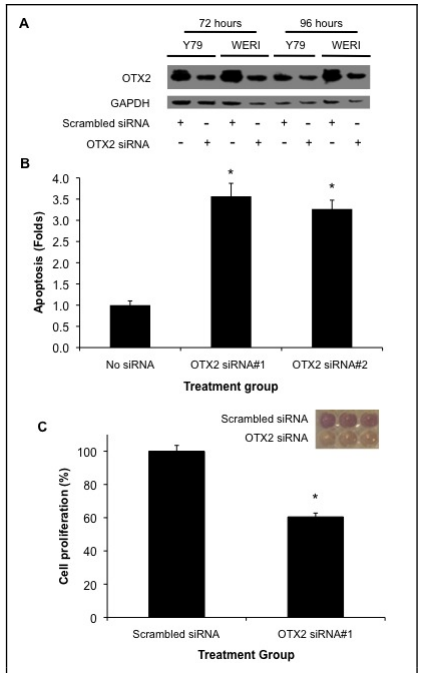

Figure 1. Inhibition of OTX2 via siRNAs at 72h (A) causes increased apoptosis by 3.5-fold (B) and decreased proliferation by 40 % (C) in retinoblastoma cell lines. (*p<0.05)

Results

1. OTX2 was frequently amplified and/or over-expressed in retinoblastoma primary tumors and cell lines

2. Knockdown of OTX2 significantly increased apoptosis and decreased cell proliferation *in vitro*

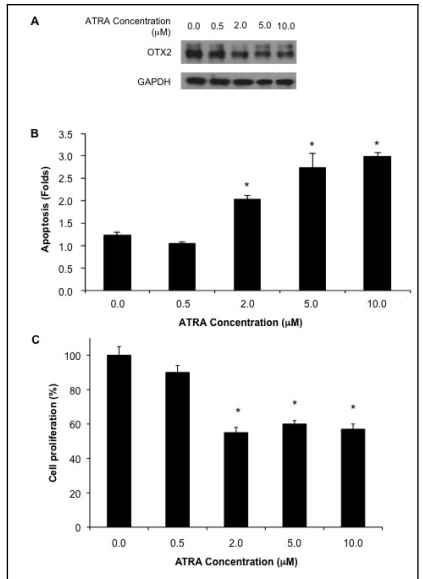

Figure 2. Pharmacologic inhibition of OTX2 at 72h with ATRA (A) causes increased apoptosis by 2-fold (B) and decreased proliferation by 40% (C) with 2 uM in retinoblastoma cell lines. (*p<0.05)

Results

3. Knockdown of OTX2 significantly decreased tumor size and growth *in vivo*

4. Inhibition of OTX2 decreased the expression of MYC and CRX, but increased the phosphorylation of RB

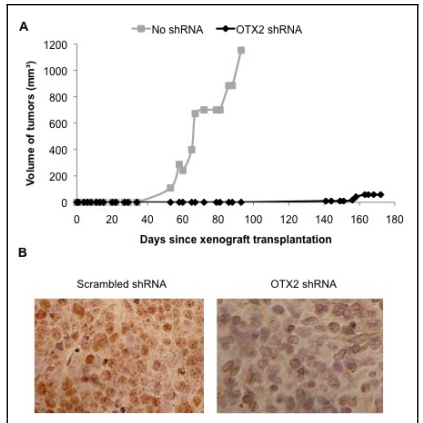

Figure 3. Inhibition of OTX2 in retinoblastoma mice xenografts by shRNA reduces tumor growth initiation and tumor size (A). OTX2-specific shRNA treatment group (black line), No shRNA control group (gray line), ($p<0.05$). IHC shows robust OTX2 staining (brown) when OTX2 is not knocked down (left) and weak OTX2 staining (purple) when OTX2 is knocked down (right) (B).

Conclusions

This study shows that the medulloblastoma oncogene *OTX2* may play an important oncogenic role in retinoblastoma via regulation of MYC and CRX and the phosphorylation of RB.

These tumors may also be amenable to therapy targeting OTX2.

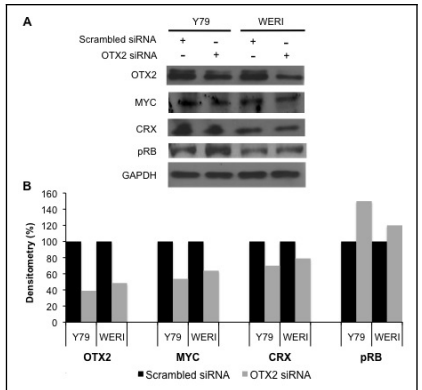

Figure 4. OTX2 inhibition by siRNA reduces OTX2, MYC, and CRX expression, but increases pRB expression in both WERI and Y79 (A). Densitometry of immunoblot results quantifies this trend (B).

References

- Adamson DC, et al. 2010. OTX2 is critical for the maintenance and progression of shh-independent medulloblastomas. Cancer Res 70: 181-191.
- Di C, et al. 2005. Identification of OTX2 as a medulloblastoma oncogene whose product can be targeted by all-trans retinoic acid. Cancer Res 65: 919-924.