ZEB1 is an Oncogenic Factor that Represses Many Tumor Suppressors in Uveal Melanoma

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Editorial

Uveal Melanoma (UM) is the most common intraocular tumor in adults, with an incidence rate of 4.3 per million in USA [1,2]. Half of the patients developdevelop metastasis preferentially to the liver and no effective treatment is available for the metastatic lesion [3,4]. UMs are transformed from the melanocytes of neural crest origin in the uveal tract with a mobile property [5] and pathologically classified as spindle (mesenchymal-like), epithelioid (epithelial-like) and mixed cell types [6] with a tendency of the epithelioid tumors to develop a metastatic disease [7]. Abnormal expression of some genes are found in UM transformation and progression including ZEB1. ZEB1 is a member of the E-box binding Transcription Factor (TF) group that includes ZEB, SNAI, and TWIST families functioning in Epithelial to Mesenchymal Transition (EMT) in carcinomagenesis [8]. This assumption was experimentally verified by our observations both in vivo where the spindle OCM1 UM cell line-grafted tumors displayed both spindle and epithelioid cell types while the epithelioid C918 UM cell line-grafted tumors only gave rise to epithelioid cell type, and in vitro where a single spindle OCM1 cell-formed organoid could generate cells of various shapes including spindle and epithelioid cell types while a single epithelioid C918 cell-formed organoid could only produce epithelioid cells [11]. Furthermore, overexpression of ZEB1 in ZEB1 hi NS C918 and knockdown of ZEB1 in ZEB1 lo NS C918 did not affect their cell morphology [11], suggesting that ZEB1 has little effect on UM cell morphology, but is a major oncogenic factor for UM progression.

Our laboratories and others’ have revealed that ZEB1 is almost undetectable in Normal Uveal Melanocytes (NUM), but states high in primary UM and even higher in Metastasized UM (MetUM), and that its expression is significantly higher in epithelioid cells than in spindle cells [11], suggesting that ZEB1 involves in UM tumorigenesis and malignant evolution. We therefore reason that initial transformation-of elongated fibroblast-like NUM to the spindle UM and then development to the epithelioid UM phenotype is delimited by a gradually increased ZEB1 expression. This assumption was experimentally verified by our observations both in vivo where the spindle OCM1 UM cell line-grafted tumors displayed both spindle and epithelioid cell types while the epithelioid C918 UM cell line-grafted tumors only gave rise to epithelioid cell type, and in vitro where a single spindle OCM1 cell-formed organoid could generate cells of various shapes including spindle and epithelioid cell types while a single epithelioid C918 cell-formed organoid could only produce epithelioid cells [11]. Furthermore, overexpression of ZEB1 in ZEB1 hi NS C918 and knockdown of ZEB1 in ZEB1 lo NS C918 did not affect their cell morphology [11], suggesting that ZEB1 has little effect on UM cell morphology, but is a major oncogenic factor for UM progression.

To assess biological functions of ZEB1 in regulation of UM progression in detail, we adopted a loss-of-function approach using shRNA to knockdown ZEB1 expression in both OCM1 and C918 UM cell lines for grafting in the vitreous of the nude mice and for molecular analyses [11]. As results, we find that knockdown of ZEB1 significantly reduces UM cell proliferation both in culture and in the grafted tumors. The cause of ZEB1-induced cell proliferation is linked to the binding of ZEB1 to a group of Cyclin-Dependent Kinase Inhibitors (CDKIs) such as P21 that prevent activation of RB1, a major tumor suppressor and cell cycle regulator [13]. More importantly, ZEB1 is observed to bind to and repress another cell cycle regulator and also a well-known UM suppressor BRCA1 associated protein 1 (BAP1) [11,14]. Secondly, we find that ZEB1 highly expresses in non- or less pigmented UM cells as it binds to the melanocyte promoter of the pigment synthesis regulator
gene **MITF** and repress its expression. We link the ZEB1-repressed MITF expression to the dedifferentiated state of UM, indicative of a more advanced stage. And again, ZEB1 is observed to bind and repress another cell differentiation facilitator and also a well-known UM suppressor **ID2** (inhibitor of DNA binding protein 2) [11,15]. It is of note that the famous carcinoma suppressor **E-cadherin** (CDH1) is hardly detectable in most primary UMs [11,15] and cell lines, and knockdown of ZEB1 has little effect on its expression [11], suggesting that ZEB1 mechanistic regulation of tumorigenesis in UM differs from carcinomas. Thirdly, knockdown of ZEB1 degrades the invasiveness of grafted UMs in the vitreous of the model animals likely by reducing tumor cell capacity to break through the healthy tissues as ZEB1 can bind to and thereby transactivate those extracellular matrix degradation enzyme genes like matrix metalloproteinase 11 (**MMP11**) and urokinase-plasminogen activator (**PLAU**) [11]. Fourthly, knockdown of ZEB1 significantly decreases mobility of UM cells in culture and reduces UM metastasis in the grafted model animals [11]. ZEB1-increased UM cell mobility is accomplished by binding to and thereby repressing the expression of the extracellular matrix protein Fibronectin 1 (**FN1**) gene, but transactivating cell migration protein profilin1 (**PFN1**) gene [11].

In addition, in melanomas of both uveal and cutaneous origins a G protein-related signaling pathway is frequently activated through a gain-of-function mutation of either large GTPase like **NRAS** and its downstream effector **BRAF** [17,18]. We also find that **BRAF** and **GNA11** and **GNAQ** are transcriptionally upregulated in UM and MetUM [11], suggesting that although **GNA11/GNAQ** or **NRAS/BRAF** mutations are often found in uveal and cutaneous melanomas, respectively [19], the overall elevated transcription levels of the above genes position UM cells in a high potential readiness for extracellular signals for cell proliferation regardless of their gain-of-function mutation status. Finally, we downloaded two sole UM microarray datasets (GSE22138 with 63 primary tumors and GSE44299 with 51 primary tumors) from National Center for Biotechnology Information (NCBI) website, and divided the primary UMs in each array into ZEB1-high and ZEB1-low groups based on their tumor ZEB1 expression levels to see if ZEB1 expression in primary UM can be used to estimate metastasis. The Kaplan-Meier survival curves of these two large cohorts show highly significant relationship between ZEB1-high group and their metastases [11], suggesting that ZEB1 expression levels can be used to predict UM patient prognosis in the clinic.

As summarized in Figure 1, we have recently demonstrated that expression of ZEB1 is positively correlated with UM malignant progression as with carcinomas, but not through EMT, which differs from carcinomas, and that its oncogenic function is realized via direct binding and repressing major tumor repressors like **CDKN1A, ID2** and **BAP1** in enhancing tumor cell dedifferentiation, proliferation, invasion and metastasis.

**Acknowledgements**

This work was supported by grants from the National Natural Science Foundation of China (Grant Numbers: No. 3087282 and No. 81072221) and the National Science Foundation of Hunan Province (14J2005) and by the Basic Research Grant of University of Louisville School of Medicine (E0819) and Research to Prevent Blindness.

**References**


