ZEB1 in Uterine Tumors

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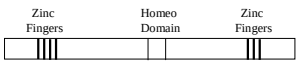
ZEB1



Binds E-box like sequences CACCTG

Has two Kruppel type-C_2H_2 zinc finger clusters located close to the N- And C-termini with 4 and three zinc-fingers respectively. A homeodomain located between the zinc finger clusters shows highest similarity to those in LIM proteins.

ZEB1 – human (other names AREB6)
δEF1 – mouse
Zfhep – rat
Zfh-1- *Drosophila*

Background on ZEB1

- ZEB1 is a transcriptional repressor or activator depending on its expression levels, conformation, and gene promoter that it is acting upon.
- is upregulated by estrogen in the chick oviduct (Chamberlain and Sanders 1999; Dillner and Sanders 2004).
- implicated in epithelial to mesenchymal transitions (Guaita et al. 2000) and represses E-Cadherin in lung and breast cancer cells (Guaita et al. 2002; Ohira et al. 2003; Eger et al. 2005).
- highly expressed in breast cancer cell lines with an invasive/metastatic phenotype compared to poorly invasive/non-metastatic cell lines (Kirschmann et al. 1999).
- is upregulated via progesterone receptors in breast cancer cells (Richer, et al. 2002).

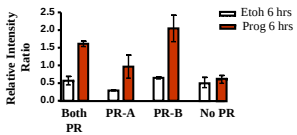


Figure 1. ZEB1 is upregulated by progesterone in T47D-derived breast cancer cell lines. Cells containing either both PR isoforms, PR-A (A), only, PR-B only (B), or no PR, were treated with 10 nM progesterone for 6 hrs. Data from Affymetrix chips regarding ZEB1 are plotted with data from triplicate independent cultures shown with error bars. Relative intensity values indicating expression level of ZEB1 were normalized by dividing by the average expression level over all treatments, thus they cluster around 1.0.

Figure 2. PR increases ZEB1 promoter activity in a progesterone-mediated manner and ER increases both basal and estrogen-mediated transcriptional activity. A segment of the ZEB1 promoter (-1000 base pairs) linked to luciferase was transfected into HeLa cells along with **A)** either no PR-B, or increasing amounts of PR-B or **B)** no ER, or increasing amounts of ER. Cells were treated with either ethanol vehicle (white) or progesterone or estrogen respectively (red).

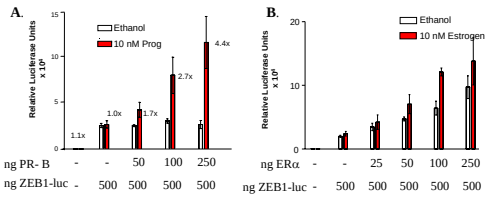


Figure 4. ZEB1 promoter activity is observed in the myometrium and stroma of the mouse uterus. X-gal staining of uteri from ZEB1/δEF1lacZ female mice (from Dr. Yushiro Higashi, Osaka, Japan) demonstrating ZEB1 promoter activity detected by x-gal staining. Mice were sacrificed at **A.** 5 week virgin, **B.** pregnancy day 5, **C.** pregnancy day 17, and **D.** Day 9 of lactation, and x-gal stained uteri were embedded in paraffin and sections stained with nuclear fast red.

Figure 5. ZEB1 is upregulated by estrogen and progesterone in mouse uterine stroma and myometrium. Wild type C57BL6 mice were ovariectomized at 4-6 weeks, rested for 2 weeks, then injected with estrogen or progesterone in sesame oil subcutaneously for 6 or 24 hours. Uteri were formalin fixed and paraffin embedded and immunostained with antibody recognizing ZEB1 (kind gift from Dr. Doug Darling) . **A)** Anti-ZEB1 antibody recognizes ZEB1 in COS cells transfected with ZEB1 cDNA expression vector and nothing in vector only or untransfected cells. **B)** ZEB1 immunostaining of paraffin embedded sections of uteri from mice treated with either vehicle only, or estrogen (E) (top) or progesterone (P) bottom for 6 or 24 hours. Uteri are shown at 40X. **C)** myometrium specifically is shown at 100X.

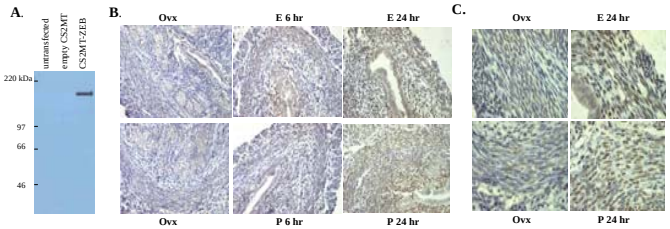


Figure 6. ZEB1 is abundant in both normal human myometrium and leiomyomas and is slightly upregulated in leiomyomas. Immunostaining of ZEB1 was performed on 14 human surgical resection specimens of leiomyomas and matched normal myometrium. **A**. In some cases ZEB1 staining was present in a higher percentage of cells and at a higher intensity in the leiomyoma compared to matched normal myometrium from the same patient, as shown in these two examples. However, the average percent cell staining was 75% in the normal myometrium with an average intensity of 3.6+ and in the leiomyoma it was 93%, 3.8+. Thus, it was difficult to determine if there was a true difference by this qualitative method. **B)** We therefore performed real-time quantitative RT-PCR with primers specific for the ZEB1 transcript from RNA isolated from 10 samples of normal myometruim and 12 leiomyosarcomas. Although not statistically significant, there may be increased ZEB1 in leiomyomas.

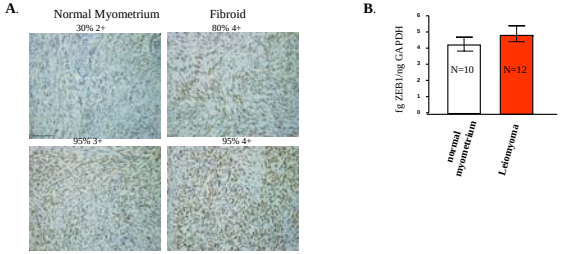


Figure 7. ZEB1 is highly expressed in leiomyosarcomas as compared to normal myometrium. We performed immunohistochemistry on paraffin embedded sections from 10 leiomyosarcomas and found that in every case the leiomyosarcomas had more intense ZEB1 staining than adjacent normal myometrium Note intense staining in regions of nuclear atypia and multinucleated tumor cells in the high grade region of the tumors. Shown are examples from 4 different leiomyosarcomas.

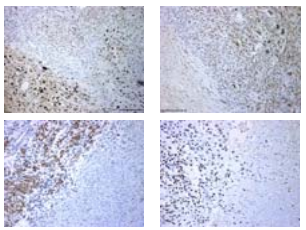


Figure 8. In the human endometrium, ZEB1 is expressed only in the stroma, and not in glandular epithelial cells. The intensity of ZEB1 staining is increased in stroma associated with endometrial cancers. ZEB1 immunostaining was examined in 12 endometrial cancers. The average intensity of staining (on a scale of 1 to 4) was 2.3 in the normal endometrial stroma and 3.6 in the stroma of matched endometrial cancers.

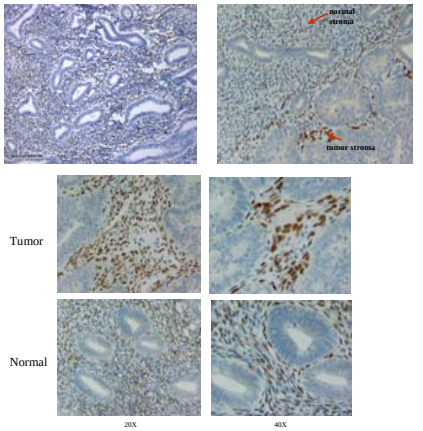
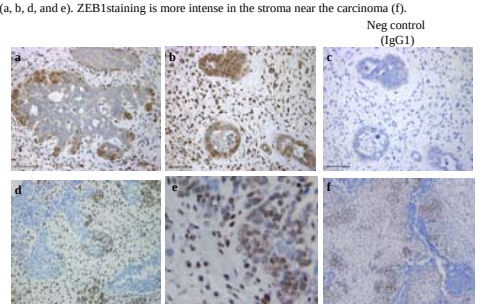


Figure 9. In malignant mixed mullerian tumors, ZEB1 is always highly expressed in the sarcomatous, and occasionally in the carcinomatous component. ZEB1 immunostaining was examined in 29 malignant mixed mullerian tumors. Interestingly, in five tumors ZEB1 was presentin the carcinomatous portion of the tumor as well as the sarcomatous component (a, b, d, and e). ZEB1staining is more intense in the stroma near the carcinoma (f).



Conclusions:

- **The ZEB1 promoter is upregulated via estrogen and progesterone receptors.**
- **ZEB1 protein is expressed in the mouse uterus, particularly in the myometrium and stroma and is upregulated by estrogen and progesterone.**
- **ZEB1 is present in normal human myometrium and may be overexpressed in leiomyomas.**
- **ZEB1 is overexpressed in leiomyosarcomas.**
- **ZEB1 is expressed in benign endometrial stroma, but not normal glandular endometrium. In endometrial cancers the intensity of staining of ZEB1 is higher in tumor stroma than adjacent normal stroma.**
- **In mixed mullerian tumors ZEB1 can be expressed in both the carcinomatous and sarcomatous components of the tumors.**
- **These observations support the role of ZEB1 in epithelial to mesenchymal transitions during tumor formation and also suggest that sex steroid hormone-mediated ZEB1 regulation could be involved in the pathogenesis of uterine tumors.**