Various EZH2 transcript variants are present in cancer cell lines

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Introduction

Enhancer of Zeste Homolog 2 (EZH2) is the catalytic subunit of Polycomb Repressive Complex 2 (PRC2), responsible for establishing suppressive H3K27me3 epigenetic mark. H3K27me3 is necessary for maintaining stem cell pluripotency and proper cellular differentiation [1,2] .

Currently, there are five experimentally confirmed EZH2 transcript variants (TV1 – TV5; Table 1). The role of TV2 and TV5 has been only recently addressed [3,4,5].

Table 1. Overview of EZH2 transcript variants - TVs (GenBank database)

TVs	Description
TV1	Longest transcript, 20 exons, 2256 nucleotides
TV2	Missing exon 4
TV3	15 nucleotides missing in exon 8
TV4	27 nucleotides missing in exon 3
TV5	27 nucleotides missing in exon 3 and missing exon 14

Results

All experiments have been performed on four cell lines - fetal fibroblasts: **IMR-90** and three cancer cell lines**: FaDu, Cal 27 and Detroit 562-** cultivated in DMEM with high (4.5 g/L) or without glucose and 10% FCS.

Three novel EZH2 transcript variants were discovered with three primer sets (Figure 1 and Table 2). The amplicons were sequenced.

- **TVinsΔ126*** variant contains 126 nucleotide from intron 14 (Figure 2A),
- **TVinsΔ82*** variant contains 82 nucleotides from intron 9 (Figure 2B),
- **TVΔexon4Δ27**** variant is a "hybrid" of TV2 and TV4 (Figure 2C),
- The sequence of **TVinsΔ126** is shown in Figure 3.
- **Western blot** analysis (Fig. 4; rabbit mAb-EZH2, CST; #5246) reveals presence of multiple, although very faint bands.
- ** present in no-glucose only; **present in both conditions*

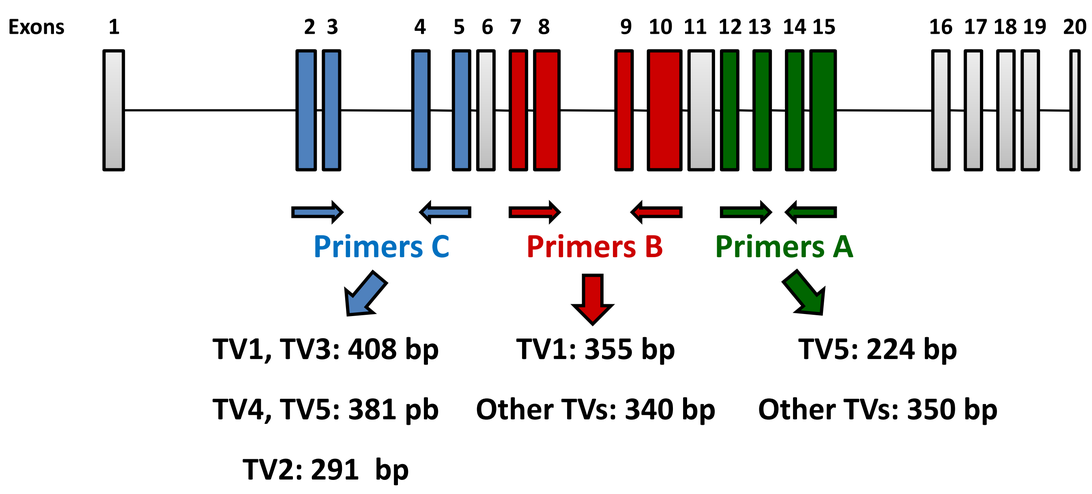

Figure 1A. Overview of the EZH2 transcript and position of primer sets

Figure 1B. Overview of the EZH2 protein coded by TV1

Table 2. Overview of newly discovered EZH2 transcript variants.

TVs	Primers	Description
TVΔexon4Δ27	**C**	27 nucleotides shorter exon 3 and missing exon 4
TV insΔ82	**B**	82 nucleotides insertion from intron 9
TV insΔ126	**A**	126 nucleotides insertion from intron 14

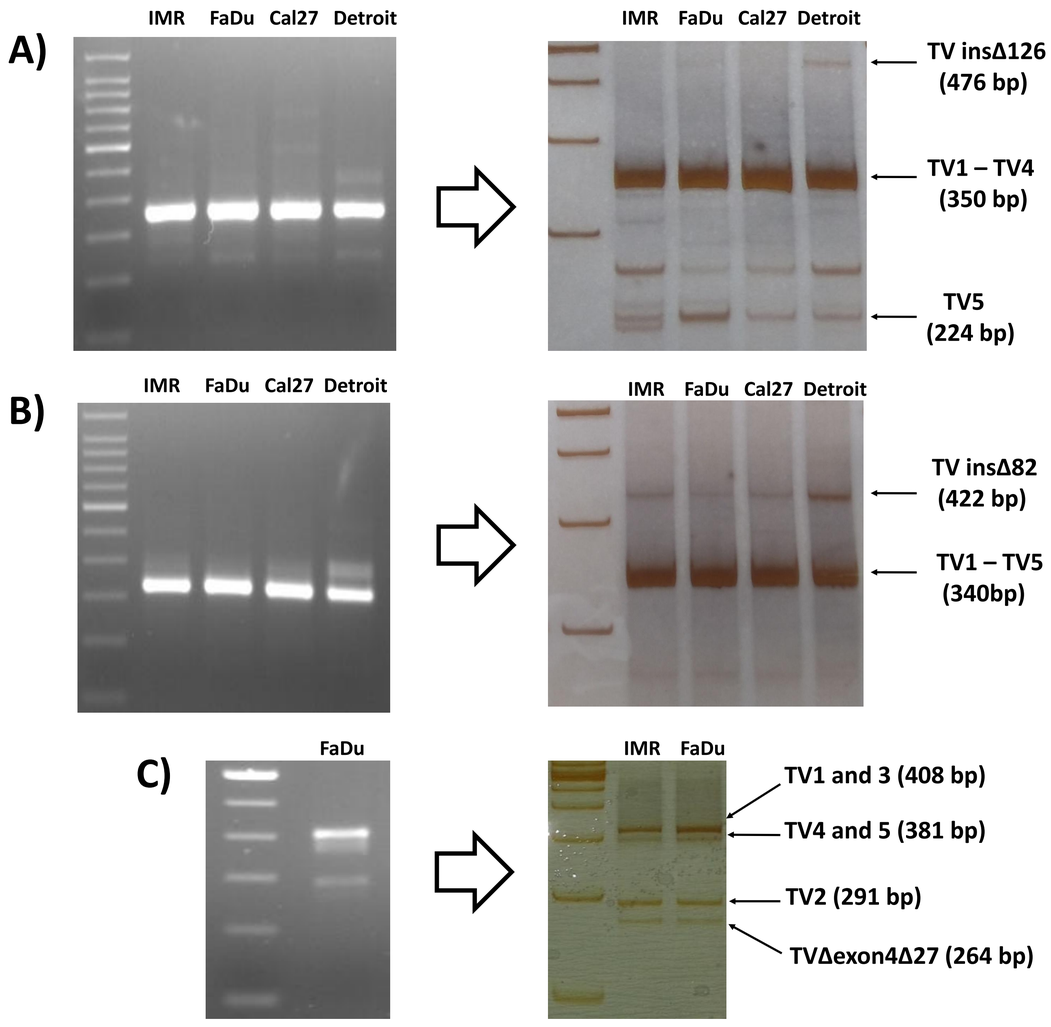

Figure 2. No-glucose PCR products on agarose and polyacrylamide (silver stained) gels, respectively. A) Primer pair A; B) primer pair B; C) primer pair C.

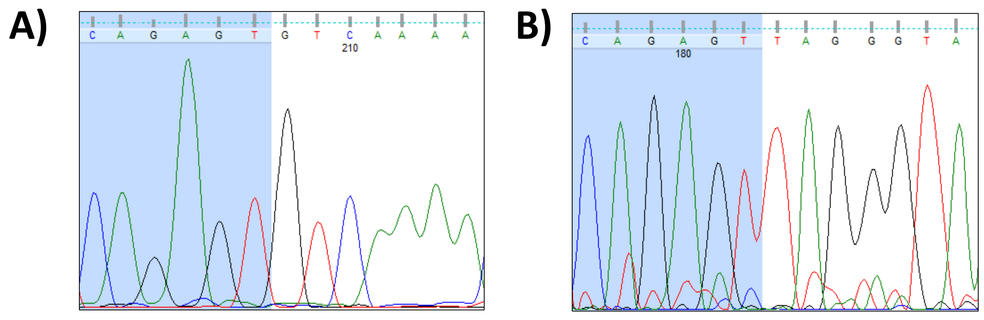

Figure 3. Comparison of dideoxy-sequencing results for TV1 (A) and TV insΔ126 (B). Blue label: 3' end of exon 14

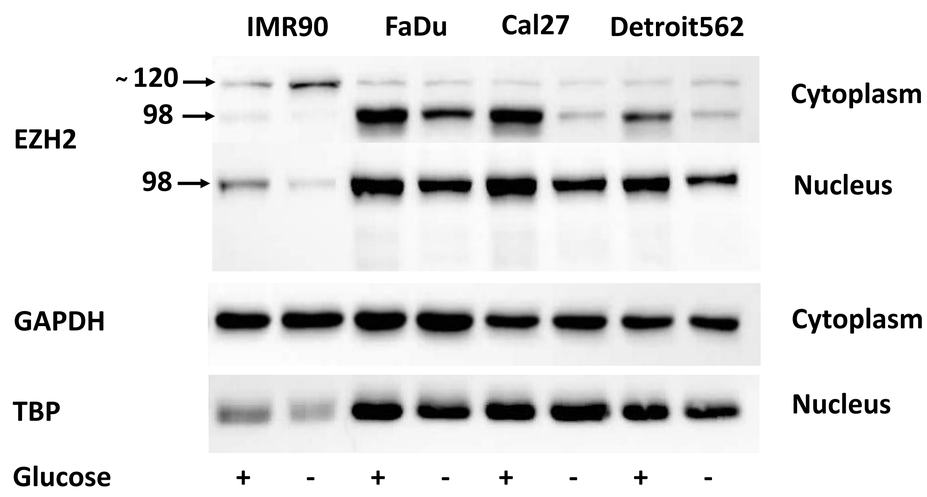

Figure 4. Western blot analysis (9% SDS-PAGE gel) of cells grown in high-glucose and no-glucose medium.

Discussion

We have discovered three new EZH2 transcript variants.

- **TVΔexon4Δ27** variant was present in all four cell lines and in both experimental conditions. Hypothetically, this variant could give rise to a protein that is 48 amino acids shorter than ˝canonical˝ isoform.
- **TV insΔ82** variant, absent in high-glucose medium, was the most abundant in Detroit 562 grown in medium without glucose. The 82 bp insertion (intron 9) results in premature **stop codon.** Hypothetically, translation of this variant would yield a protein that is 334 amino acids long (38,9 kDa).
- **TV insΔ126** variant, absent in high-glucose medium, seems to be present only in Detroit 562 grown in medium without glucose. The 126 bp insertion (intron 14) also results in premature **stop codon.** Translation of this variant would, in theory, result in a protein composed of 559 amino acids (64,1 kDa).
- Western blot analysis has revealed multiple faint bands, most likely corresponding to multiple EZH2 protein isoforms present in samples.

Acknowledgement

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