EXPLORING THE ROLE OF LONG NON-CODING RNA IN ORAL SQUAMOUS CELL CARCINOMA DEVELOPMENT AND THERAPY

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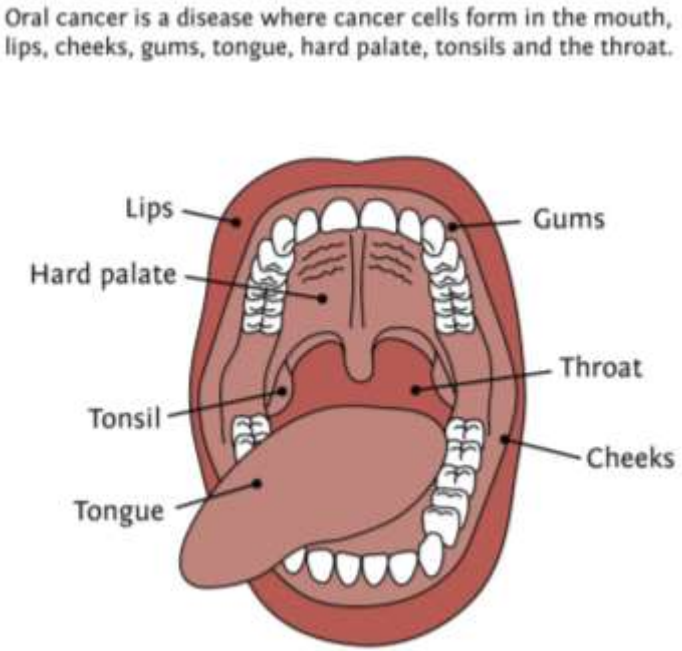
Contents

Oral Squamous Cell Carcinoma and Long non coding RNA (lncRNA)



❑**Oral squamous cell carcinoma (OSCC) or Oral Cancer** is the most prevalent type of head and neck cancer that arises in the tongue, lips, and floor of the mouth.

❑Globally, OSCC is the sixth most common type of cancer with India contributing to almost one-third of the total burden.

❑Despite the advancement of treatment, the overall survival rate is around 50% and even worse in cases of metastasis.

•**LncRNAs** are a subclass of the non-coding RNA which are more than 200 nucleotides in length and mainly transcribed by RNA polymerase II.

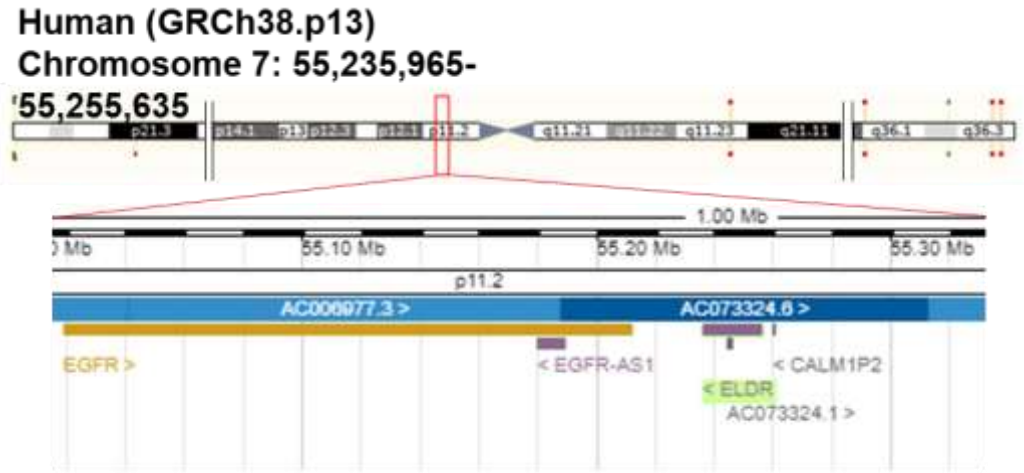
•LncRNAs interact with microRNAs to weaken regulations of miRNAs on mRNAs, directly or indirectly interact with DNAs, RNAs, or proteins thereby regulating cellular homeostasis.

•Encouraging evidence shows a strong association of lncRNAs with different types of cancers including OSCC

•The lncRNA MALAT1, HOTAIR and CCAT1 are in clinical trials for head and neck cancer, thyroid and colorectal cancer diagnostic biomarkers studies respectively.

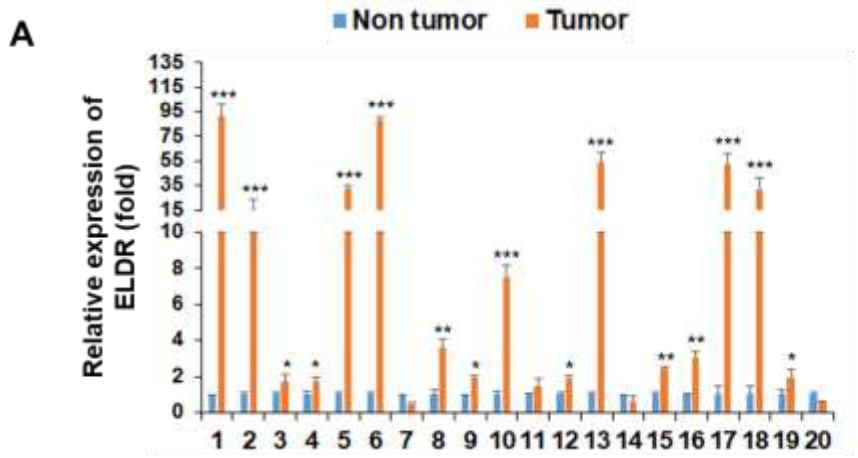
Identification of lncRNA in Oral Squamous Cell Carcinoma samples (OSCC)

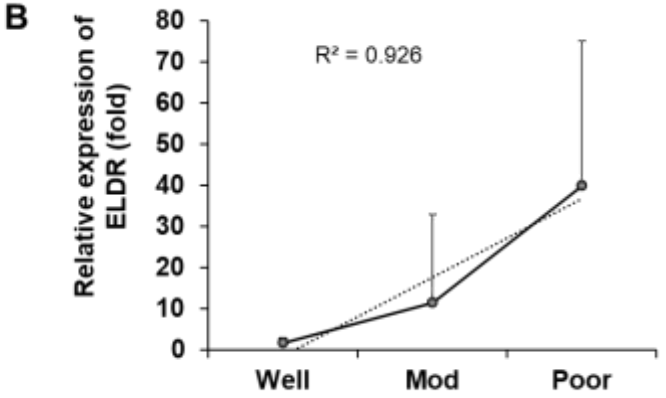
A

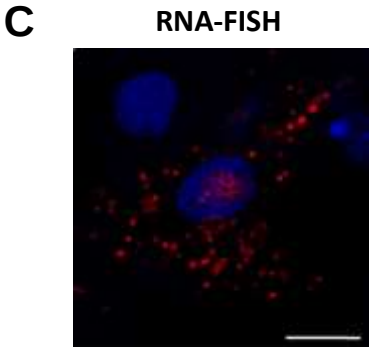


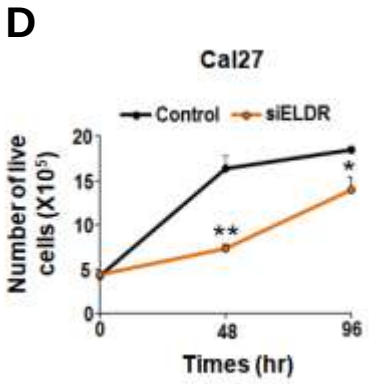
➢**EGFR long non-coding downstream RNA (ELDR): a novel lncRNA**

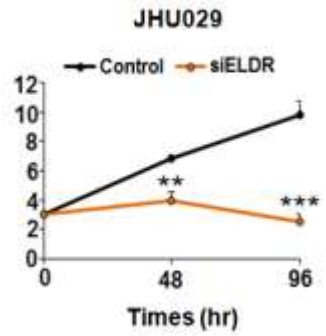
ELDR is high in oral cancer patients and regulates EGFR expression

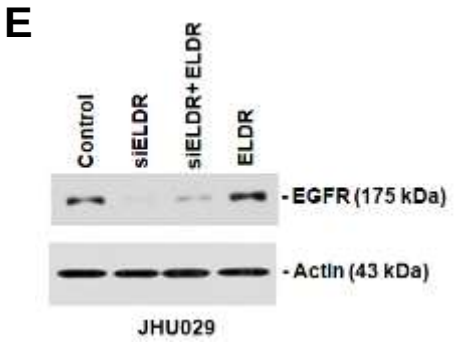


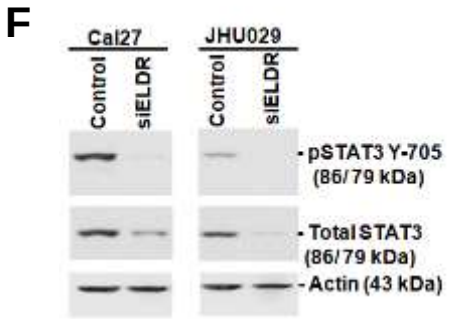


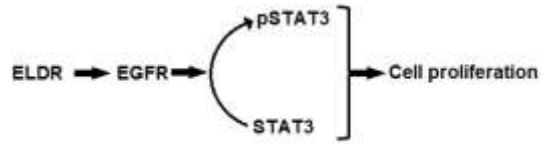










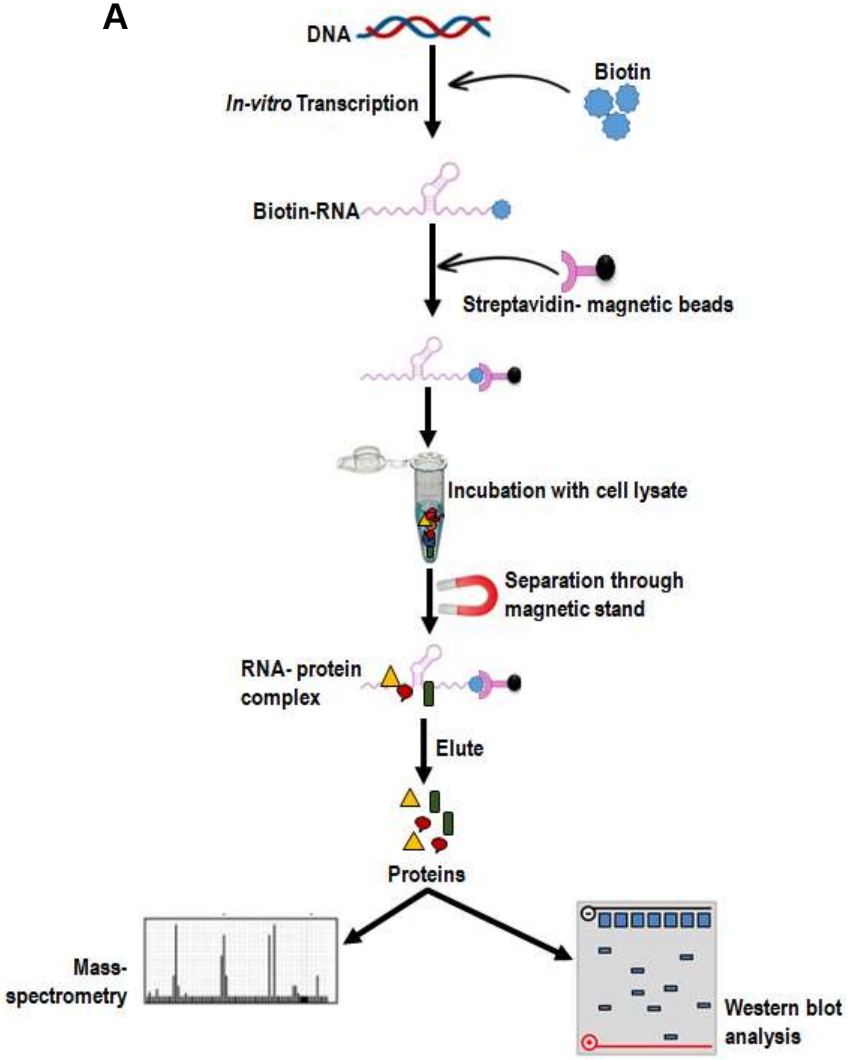


Q: How does ELDR regulate EGFR expression?

- **Hypothesis 1:** ELDR may directly regulate EGFR by physical interaction
- **Hypothesis 2:** ELDR may indirectly regulate EGFR by some interacting partner
- **Hypothesis 3:** ELDR may indirectly regulate EGFR through interaction with miRNA

ELDR interacts with interleukin enhancer binding factor-3 (ILF3) in OSCC

A



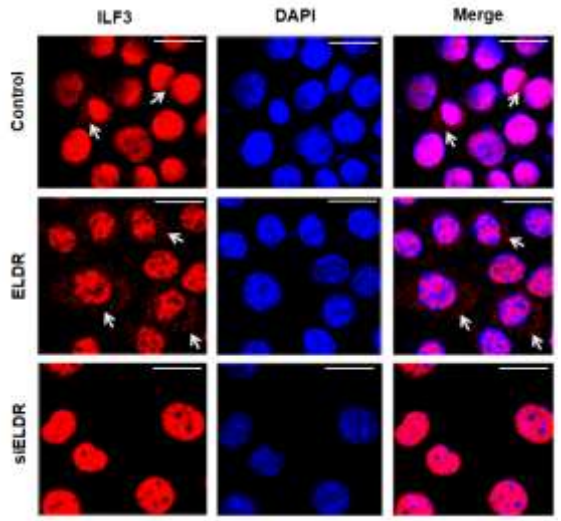
B

ELDR RNA pull-down and mass-spec analysis (top 10 proteins are shown)

Proteins	JHU022 Fold	Cal27 Fold
Serine/arginine repetitive matrix protein 2 (SRRM2)	121.7	128.25
Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2)	91.2	72.75
Interleukin enhancer-binding factor 3 (ILF3)	50.98	32.45
Ribosomal RNA processing protein 1 homolog B (RRP1B)	49.75	57.25
Zinc finger RNA-binding protein (ZFR)PE=1 SV=2	47.25	63.25
ATP-dependent RNA helicase DDX18	45.75	48.16
Spermatid perinuclear RNA-binding protein (STRBP)	45.75	45.25
ATP-dependent RNA helicase DDX50	44.5	41.25
E3 ubiquitin-protein ligase TRIM56	44	45.25
Serine/arginine repetitive matrix protein 1 (SRRM1)	43.75	62

C RNA pull-down -WB analysis

D

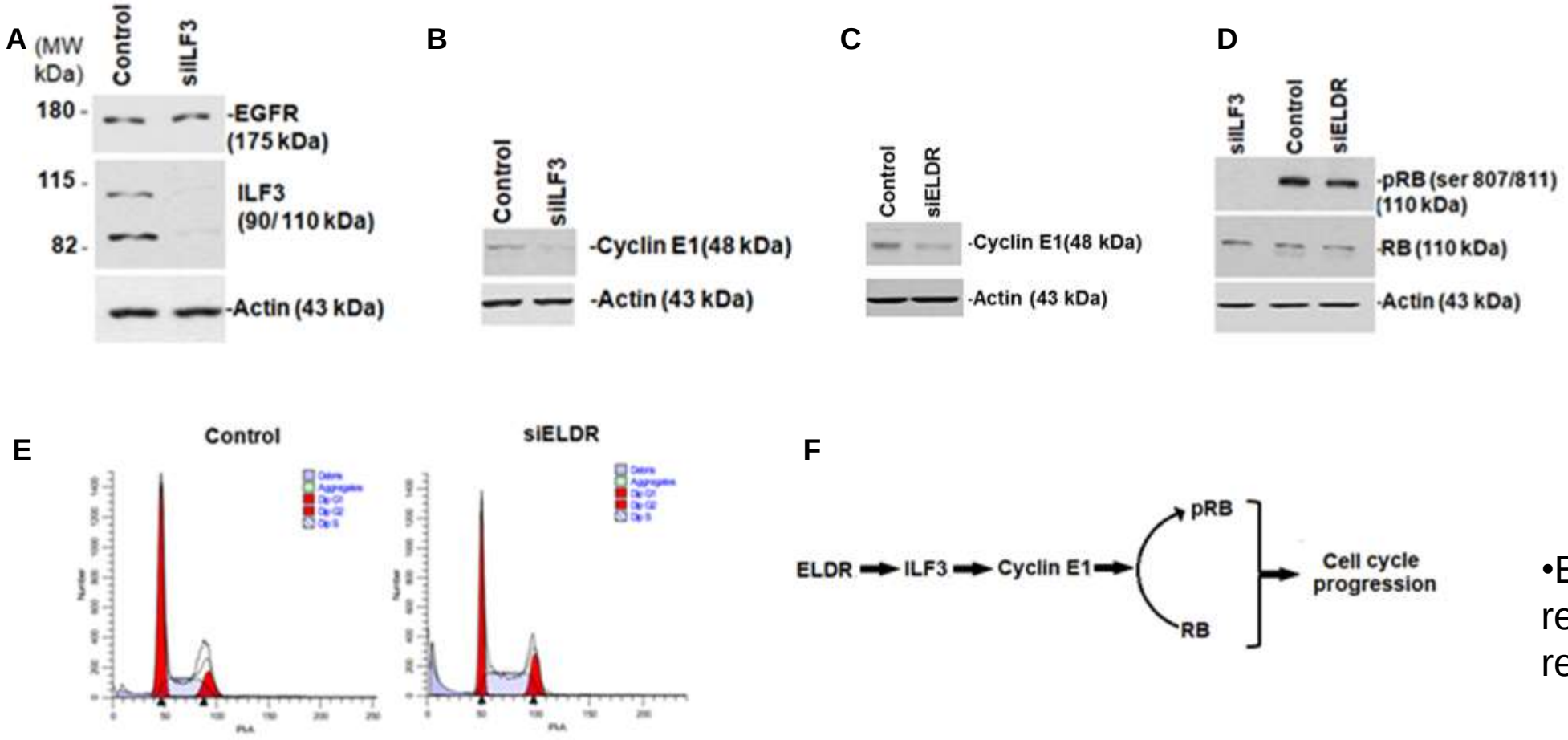


➢ ELDR does not interact with EGFR.

•**ILF3** is a DNA and RNA binding protein, functions as a transcriptional activator, regulates mRNA stability.

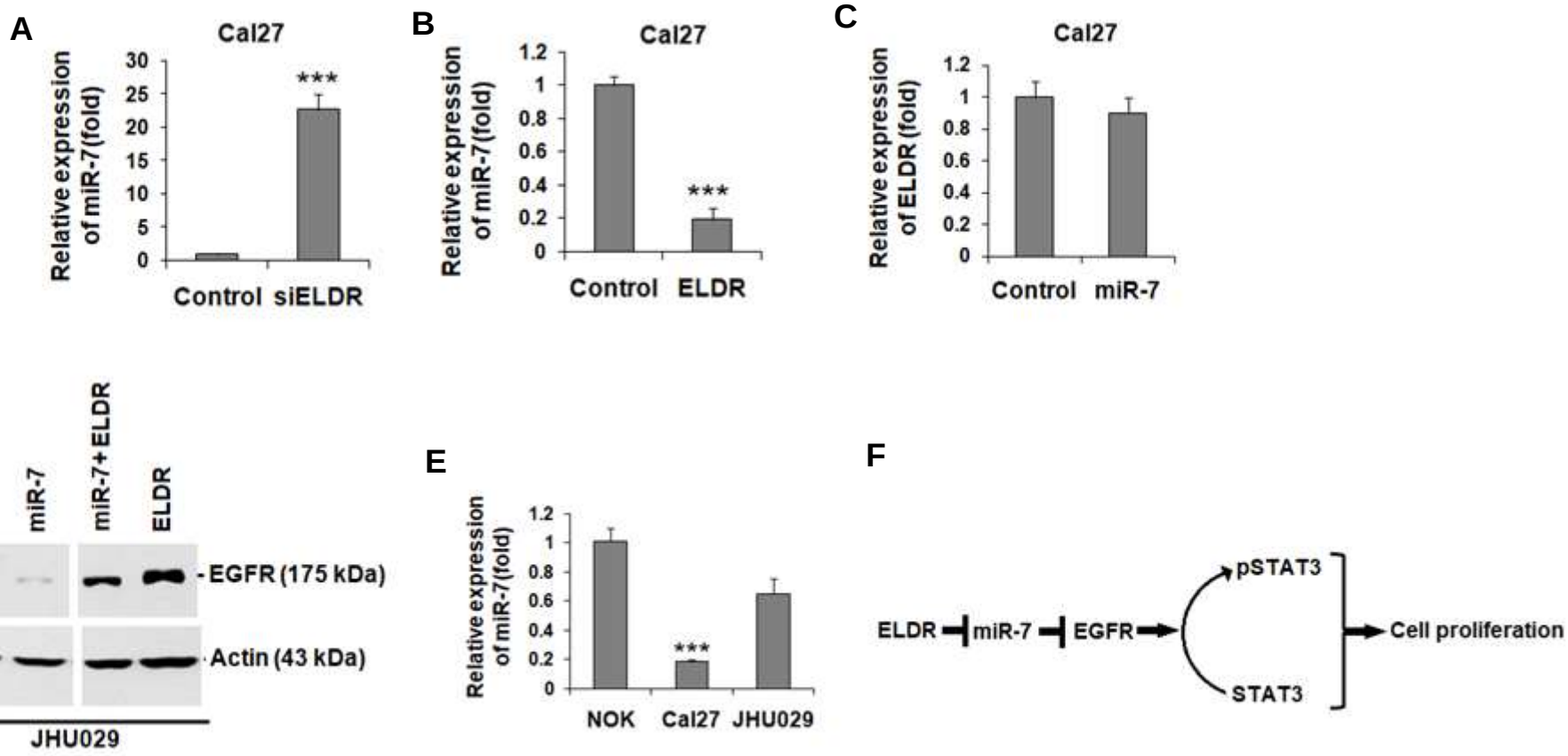
•ILF3 stabilizes EGFR and Cyclin E1 mRNA by binding at 3'UTR in cancers

ELDR -ILF3 interaction regulates Cyclin E1, not EGFR, resulting in progression of cell cycle in oral cancer cells

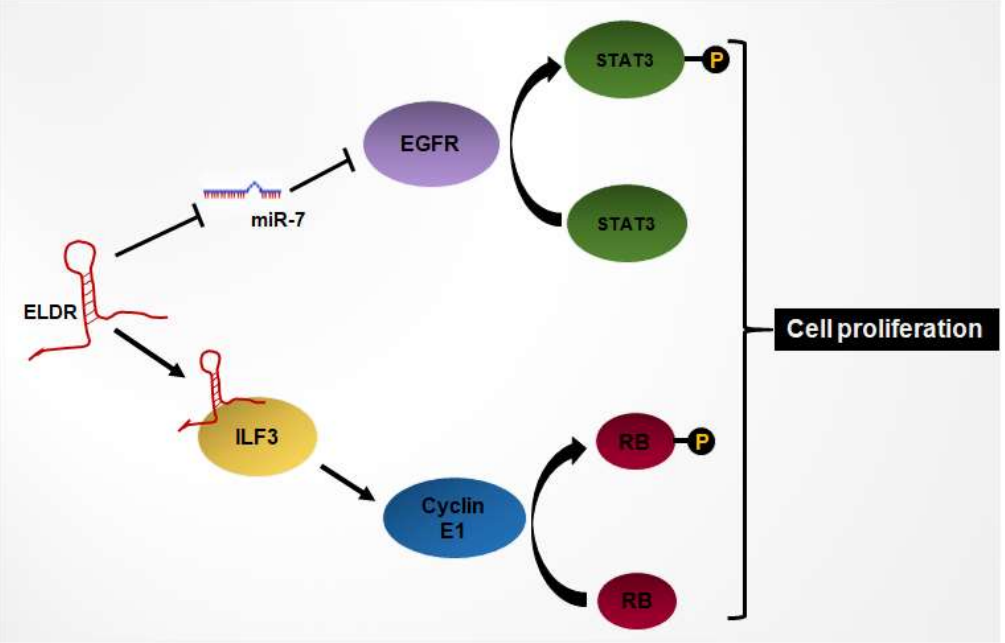


- ELDR-ILF3 axis is not responsible for EGFR regulation in OSCC
- ELDR-ILF3 axis regulates Cyclin E1 in OSCC

ELDR induces EGFR expression through inhibition of miR-7



Summary 1



ELDR induces EGFR signaling by inhibiting miR-7, and interacts with ILF3 resulting induction of ILF3- Cyclin E1 signaling in oral cancer cells. *Sharp arrow: induction/ activation, blunt arrow: inhibition*

- ELDR expression is high in oral cancer patient samples and cell lines
- ELDR induces oral cancer cell proliferation
- ELDR induces EGFR signaling by inhibiting miR-7
- ELDR interacts with ILF3 and sequesters it in cytoplasm and thereby induces Cyclin E1 mediated cell cycle progression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Long non-coding RNA ELDR enhances oral cancer growth by promoting ILF3-cyclin E1 signaling

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Author Information

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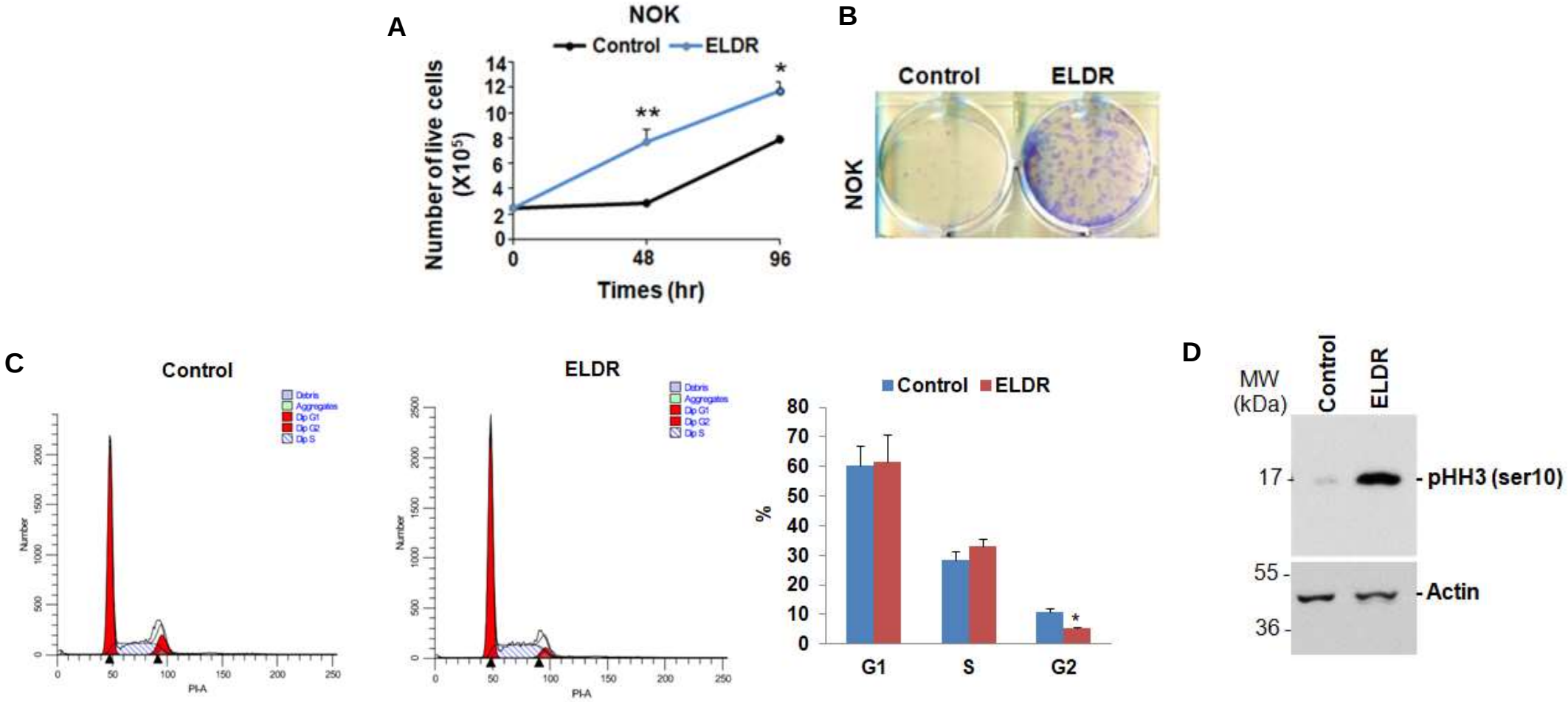
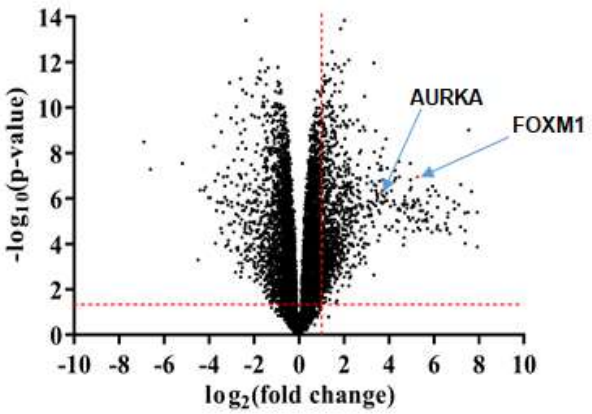
Does ELDR play as a driver gene and can transform normal oral epithelial cells?



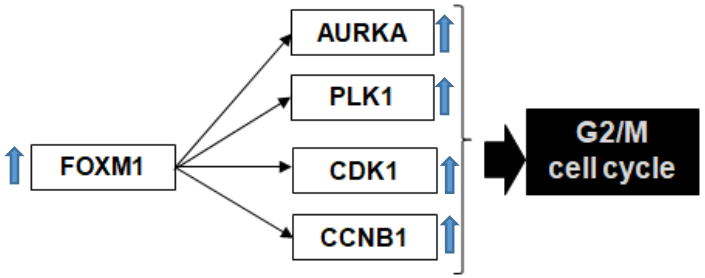
- ELDR is stably over expressed in Normal oral keratinocytes (NOK)
- NOK-ELDR showed increase in proliferation, colony formation ability and changes in G2/ M phage

ELDR induces G2/M through CTCF-FOXM1-AURKA signaling in NOK-ELDR cells

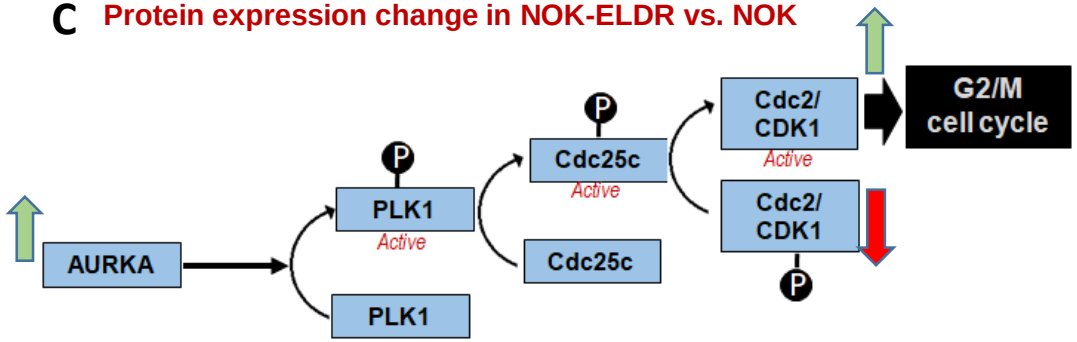
A RNA seq: NOK-ELDR vs. NOK



B Changes in G2/M phase gene in RNA and protein: NOK-ELDR vs. NOK

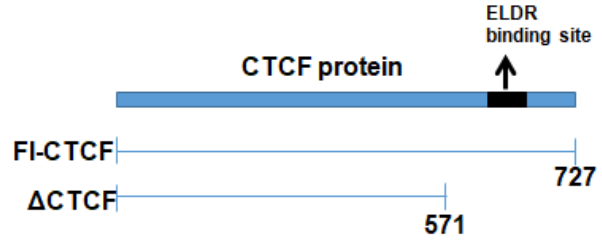


C Protein expression change in NOK-ELDR vs. NOK



D Mass Spec analysis of ELDR-RNA pull down lysates

	JHU022			Cal27		
	ELDR Anti-sense	ELDR Sense	Fold	ELDR Anti-sense	ELDR Sense	Fold
CTCF	1	16.5	16.5	1	14.5	14.5



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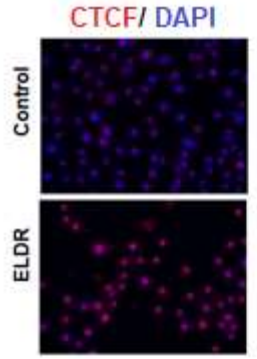

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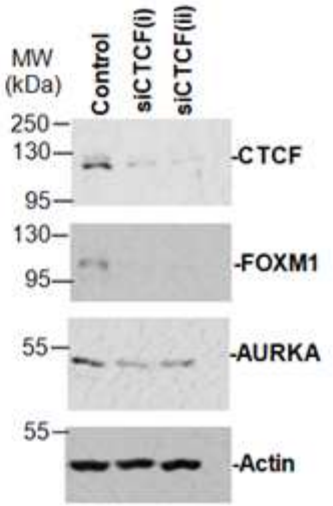
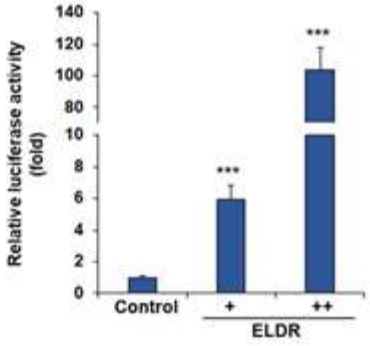
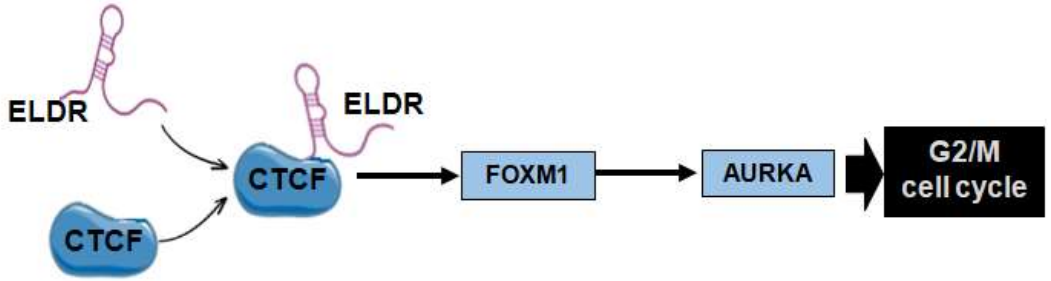


G Luc-assay: FOXM1 promoter activity



- ELDR interacts and stabilizes CTCF in regulation of FOXM1-AURKA

Summary 2



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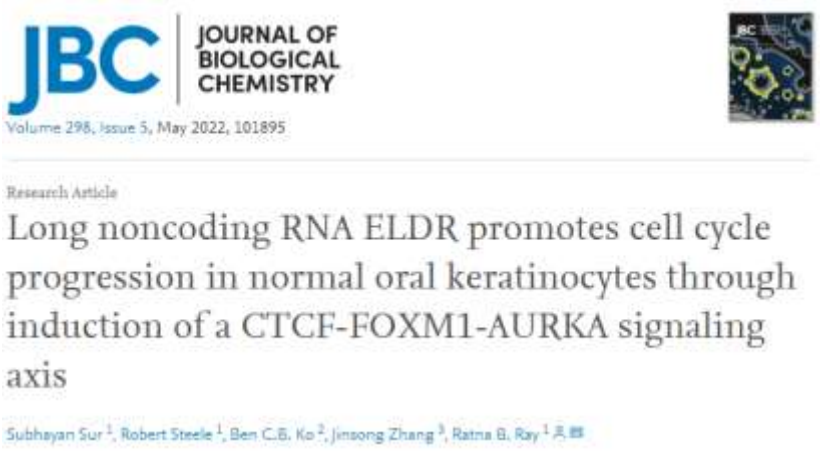
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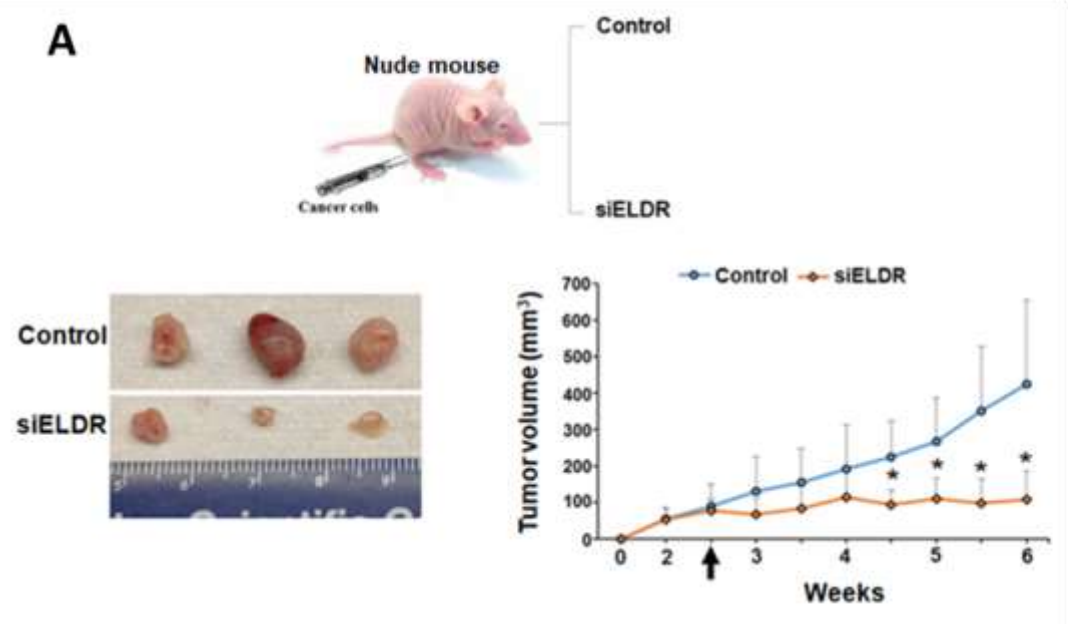
Research Article

Long noncoding RNA ELDR promotes cell cycle progression in normal oral keratinocytes through induction of a CTCF-FOXM1-AURKA signaling axis

Subhayan Sur [1], Robert Steele [1], Ben C.B. Ko [2], Jinsong Zhang [3], Ratna B. Ray [1]

- Normal oral keratinocytes (NOKs) acquire selective growth advantage when the ELDR is solely over expressed.
- ELDR over expression induces G2/M phase cell cycle progression in the NOK.
- ELDR interacts with CTCF thereby transcriptionally activates FOXM1.
- ELDR/ CTCF/ FOXM1 induces AURKA signaling resulting G2/M cell cycle progression.

Therapeutic role of ELDR in *in-vivo* Oral cancer models



EMBO Rep. 2020 Dec 3;21(12):e51042.

- Intratumor injection of siELDR regresses OSCC cell xenograft and PDX tumor in mice

Conclusion

The lncRNA ELDR plays important role in development of OSCC, and targeting the gene has potential therapeutic importance

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