**Abstract ID: -** 40

**Abstract Topic: -** Cancer

**Abstract Title: -** Super enhancer loci of EGFR regulate EGFR variant 8 through enhancer RNA and strongly associate with survival in HNSCCs

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**Aims: -** Epidermal growth factor receptor (EGFR) has been shown to be overexpressed in human cancers due to mutation, amplification, and epigenetic hyperactivity, which leads to deregulated transcriptional mechanism. Among the eight different EGFR isoforms, the mechanism of regulation of full-length variant 1 is well-known, no studies have examined the function & factors regulating the expression of variant 8. This study aimed to understand the function of EGFR super-enhancer loci and its associated transcription factors regulating the expression of EGFR variant 8 in oral carcinogenesis.

**Methods: -** We examined 48 OSCCs and 8 normal tissues for gene expression using RT-qPCR. Visualized the regulatory elements and epigenetic modifications and chromatin loop formation by UCSC. Analysis of eRNA profiles, eRNA/Hi-C interactions, and eRNA-TF factors was performed using The Cancer eRNA-Atlas.

**Results: -** Overexpression of EGFR variant 8 and its transcription was more prevalent than variant 1 and positively correlated with the EGFR-AS1 expression in oral cancers. Notably, EGFR variant 8 overexpressed patients showed shorter overall survival than variant 1 and had a greater connection with other clinical traits than patients with overexpression of variant 1. The TCGA profile further revealed that EGFR variant 8 is a significant isoform that is dysregulated in many malignancies than variant 1. GeneHancer and Hi-C analysis showed a clustered interactions between CE1, CE2, and EGFR-AS1 which regulates expression of both EGFR-eRNA and EGFR variant 8. The TCGA-eRNA analysis showed the enrichment of eRNA-specific marks POL2 signal, DNase I hypersensitivity, H3K27ac, H3K4me1, H3K4me3 in CE2 region may facilitates EGFR-eRNA synthesis by employing CE1 as an promoter. This was further supported by strong positive correlation of EGFR-eRNA with variant 8 expression. Moreover, SNAI2 transcription factor likely to

modulate EGFR-AS1 and EGFR-eRNA expression with YY1 acting as a bridging complex between EGFR-eRNA, EGFR-AS1 and EGFR variant 8. We show for the first time that novel EGFR variant 8 was significantly overexpressed than well-known EGFR variant 1 in OSCC, HNSCC and other malignancies. Further, the preference towards high-level expression of EGFR variant 8 over variant 1 is due to the presence of multiple eRNA loci in intron 1 of EGFR variant 8 and its close proximity of eRNA loci to EGFR variant 8's own promoter.

**Conclusions: -** Our findings show that EGFR variant 8 and its transcriptional regulation by eRNAs may provide a rationale for targeting RNA splicing in combination with targeted EGFR therapies in OSCCs.

**Keywords: -** Overexpression of EGFR variant 8 and its transcription was more prevalent than variant 1 and positively correlated with the EGFR-AS1 expression in oral cancers. Notably, EGFR variant 8 overexpressed patients showed shorter overall survival than variant 1 and had a greater connection with other clinical traits than patients with overexpression of variant 1. The TCGA profile further revealed that EGFR variant 8 is a significant isoform that is dysregulated in many malignancies than variant 1. GeneHancer and Hi-C analysis showed a clustered interactions between CE1, CE2, and EGFR-AS1 which regulates expression of both EGFR-eRNA and EGFR variant 8. The TCGA-eRNA analysis showed the enrichment of eRNA-specific marks POL2 signal, DNase I hypersensitivity, H3K27ac, H3K4me1, H3K4me3 in CE2 region may facilitates EGFR-eRNA synthesis by employing CE1 as an promoter. This was further supported by strong positive correlation of EGFR-eRNA with variant 8 expression. Moreover, SNAI2 transcription factor likely to modulate EGFR-AS1 and EGFR-eRNA expression with YY1 acting as a bridging complex between EGFR-eRNA, EGFR-AS1 and EGFR variant 8. We show for the first time that novel EGFR variant 8 was significantly overexpressed than well-known EGFR variant 1 in OSCC, HNSCC and other malignancies. Further, the preference towards high-level expression of EGFR variant 8 over variant 1 is due to the presence of multiple eRNA loci in intron 1 of EGFR variant 8 and its close proximity of eRNA loci to EGFR variant 8's own promoter.