analysis reveals that the majority of differentially methylated genes in the enhancer region are involved in the cancer and focal adhesion pathway. In addition, the pathways in cancer and PI3K-Akt signaling are significantly enriched in the differentially methylated open chromatin loci. Significant differentially methylated enhancer and open chromatin loci, OPLAH cg26256223 (hypermethylated open chromatin) and LYN cg08621168 (hypomethylated enhancer) were selected for further validation. qPCR analysis further confirmed the decrease of OPLAH gene expression level and vice versa for the LYN gene.

Conclusions This is the first insight on the enhancers and open chromatin methylation profile in Malaysian CRC patients. The new knowledge from this study can be utilized to further increase our understanding of CRC methylinics, particularly on the enhancers and open chromatin. The functional roles of OPLAH cg26256223 and LYN cg08621168 warrant future investigations.

IDDF2019-ABS-0307 LONG NON-CODING RNA TMPO-AS1 REGULATES OESOPHAGEAL SQUAMOUS CELL CARCINOMA METASTASES THROUGH ACTIVATING GLI1 BY MAINTAINING LAP2A EXPRESSION

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Background Long non-coding RNAs (lncRNA) have been found to play important regulatory roles in cancer development and progression. However, functional lncRNAs and their downstream mechanisms remain largely unknown of oesophageal squamous cell carcinoma (OSCC) metastases. We aimed to identify lncRNAs that regulate OSCC metastases and investigate their downstream mechanisms.

Methods Small interfering RNA library was built of the top 50 overexpressed lncRNAs in OSCC according to TCGA database. Transwell migration assay was performed to identify the lncRNA that markedly affected cell migration. TMPO-AS1 expression was validated by qPCR in patient tissues and OSCC cell lines. Gain and loss of function of TMPO-AS1 were performed in transwell migration and invasion assays in vitro. Lung and lymph node metastases models were built with short hairpin RNA knockdown in vivo. RNA pull-down and RNAase protection assay (RPA) followed by qPCR analysis identified the RNA-RNA interaction. Downstream mechanisms were examined by regular molecular biological methods.

Results We identified TMPO-AS1 as a key regulator of OSCC metastases. TMPO-AS1 expression was upregulated in OSCC tumor tissues compared to adjacent normal tissues and positively correlated with the mRNA expression of TMPO (LAP2), its sense coding gene. Knockdown of TMPO-AS1 significantly inhibited OSCC cells migration and invasion in vitro and attenuated metastases in two different animal models in vivo. Overexpression of TMPO-AS1 showed contrary effects. RNA pull-down identified the interaction between TMPO-AS1 mRNA and LAP2a mRNA. RPA assay further confirmed TMPO-AS1’s protective effect on LAP2a mRNA. Western blotting found that knockdown of TMPO-AS1 decreased the expression of LAP2a without affecting LAP2b. Ectopic expression of LAP2a after TMPO-AS1 knockdown rescued the adverse effect on cell migration and invasion. By regulating the expression of its sense coding gene LAP2, TMPO-AS1 maintained the levels of LAP2a, which in turn activated Hedgehog signaling transcription factor GLI1 and its downstream target SNAIL, therefore promoting OSSC progression.

Conclusions TMPO-AS1 acts as an essential regulator in OSSC metastases by interacting with LAP2a mRNA and maintaining its levels, which activates the LAP2a-GLI1-SNAIL axis and facilitates OSSC metastases.