1722P: YBX1 Integrates the Oncogenic PI3K/mTOR Signalling to Regulate Subtype-specific Cancer Cell Fitness

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INTRODUCTION

1. Advanced head and neck cancer (HNC) is highly resistant to therapy with a five-year overall patient survival of less than 20% (approximately 10-months median overall survival); 10% of HNC patients present with metastatic disease at initial diagnosis with an additional 20-30% after first-line treatment.

2. Frequent dysregulation of the phosphatidylinositol 3-kinase (PI3K) signalling pathway is found in HNC tumours. Over 40% of patients present with amplification of the PIK3CA gene (encoding the catalytic subunit of the PI3K complex) and up to 10% with PIK3CA gain-of-function mutations.

3. Invasive cells at the leading edge of combined malignant-basal HNC undergo partial epithelial to mesenchymal transition (pEMT) while the pEMT program within the tumour core is suppressed. In HNC patients, pEMT serves as a predictive biomarker of nodal metastases, lymphovascular invasion and extranodal extension.

4. The transcription/translation Y box binding protein 1 (YBX1) is a prognostic biomarker for subtype-specific survival in HNC and is highly expressed in high-grade HNC cells at the tumour invasive front.

HYPOTHESIS: Molecular factors that regulate pEMT downstream of the PI3K/mTOR signalling could serve as therapeutic targets against metastatic HNC.

RESULTS

The pEMT signature emerges in epithelial cancer cells and inversely correlates with PI3K pathway activation in patients.

Figure 2: The pEMT Signature Inversely Correlates with PI3K Pathway Activation in Basal and Mesenchymal HNC Patient Subtypes.

A) The EMT ssGSEA score is significantly higher in mesenchymal compared to basal HNC subtype. The results are presented as mean ± SEM with ***p-value < 0.0001. B) Significant negative correlation between ssGSEA scores of the EMT and PI3K/ACTK/mTOR gene sets from the basal and mesenchymal HNC samples (Spearman’s correlation). C) UMAP plot of single cells from 6 primary and metastatic tumours assigned to 13 clusters by the nearest-neighbour method. The clusters were assigned to the indicated cell types by differentially expressed genes. D) Box plots depict ssGSEA scores of EMT and PI3K/ACTK/mTOR hallmark gene sets and their significant association with the G1, G2/M and S phases of the cell cycle. Comparisons between groups were performed using Kruskal-Wallis t test and unpaired two samples Wilcoxon test.

The YBX1 factor links the PI3K/mTOR signalling to pEMT in specific HNC subtypes.

Figure 3: Active PI3K Signalling Induces Phosphorylation of YBX1 in Basal-like SCC25 Cells.

A) Heatmap showing EMT markers and PI3K/ACTK/mTOR signalling gene expression in mesenchymal-like SCC15 and basal-like SCC25. B) Bioluminescence imaging of five animals at 38 days post-arthropod xenograft, showing a subtype-specific distribution of bioluminescence in SCC15 mice. C) WB analyses of YBX1 and pYBX1 in the HNC cell lines. D) In vitro YBX1 and pYBX1 expression in cells treated with 100 ng/mL EGF or 20% FBS for 30 min. EGF and FBS treatments induce phosphorylation of YBX1 in SCC25 but not in SCC15.

CONCLUSION

1. A mutually exclusive interplay between PI3K-mediated cell proliferation and pEMT-imitated invasion is identified in HNC basal and mesenchymal subtypes.

2. PI3K-dependent phosphorylation of YBX1 is a limiting factor of the proliferation-to-invasion switch.

3. Shifting the mesenchymal to a basal subtype may sensitize HNC to PI3K inhibitors and represent a promising therapeutic opportunity against metastatic HNC.

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Figure 4: The PI3K-pYBX1 Axis in Basal and Mesenchymal HNC Subtypes Predicts Patient Prognosis and Inversely Correlates with FDPN Expression.

A) The correlation of ssGSEA scores between the hallmark EMT gene set and YBX1 (R = 0.2187, p-value=0.091) or phospho-YBX1 (R = −0.3438, p-value<0.01) from basal and mesenchymal HNC samples (n = 72, Spearman’s correlation). B) Kaplan-Meier survival analysis of HNC patients stratified based on the protein level of YBX1 or phospho-YBX1. The survival of the pYBX1Hi cohort was significantly superior to that of pYBX1Lo patients (log-rank test). C) Lymph node stage from the PI3K-pYBX1 axis in basal and mesenchymal HNC samples (Spearman’s correlation). D) UMAP plot of single cells from 6 primary and metastatic tumours assigned to 13 clusters by the nearest-neighbour method. The clusters were assigned to the indicated cell types by differentially expressed genes. E) Box plots depict ssGSEA scores of EMT and PI3K/AKT/mTOR hallmark gene sets and their significant association with the G1, G2/M and S phases of the cell cycle. Comparisons between groups were performed using Kruskal-Wallis t test and unpaired two samples Wilcoxon test.

Figure 5: YBX1 distinct roles in HNC proliferation and invasion. Schematic diagram depicting the PI3K-pYBX1 proliferation axis in HNC. Oncogenic activation of PI3K signalling induces YBX1 phosphorylation and nuclear translocation to promote cellular proliferation. Inactive PI3K signalling correlates with the cytoplasmic accumulation of YBX1 and the induction of pEMT protein translation.