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The Role of CBX5 in Head and Neck Cancer Cell Phenotypic Expression

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The Role of CBX5 in Head and Neck Cancer Cell Phenotypic Expression

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Summary

- Head and neck squamous cell carcinomas (HNSCCs) have remained one of the common and lethal cancers around the world.
- HNSCC rapidly spread via the lymphatics system, leading to a higher percentage of initial late-stage diagnoses and poor prognoses for patients.
- CBX5 is downregulated highly metastatic breast cancers and may repress metastatic phenotypes
- It is unclear what role is may have in HNSCC, or which specific functions of CBX5 drive its antimetastatic role.
- We aim to map molecular changes to phenotypic expression in order to provide better diagnostic prognoses for different HNSCC cell types as well as possible pharmacologic targets for treatment.

HYPOTHESIS: Heterochromatin binding by CBX5, but not dimerization or ligand activation, is necessary to suppress in vitro metastasis in HNSCC cell lines.

Regional Metastasis Significantly Decreases Survival and is Associated with Negative Treatment Outcomes

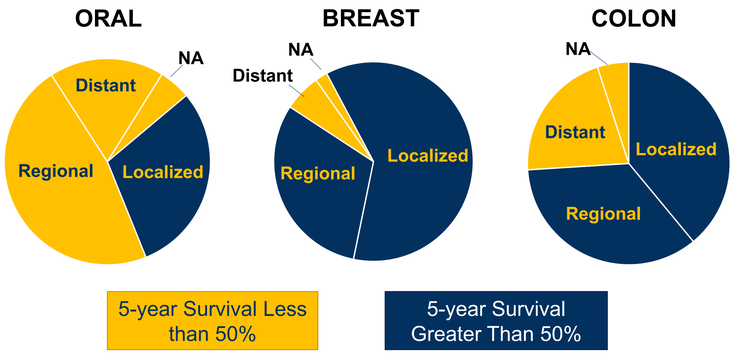


Figure 1. HNSCC rates are on the decline overall as are many cancers, however, the survivability of HNSCC has not significantly changed over time unlike other cancer subtypes.

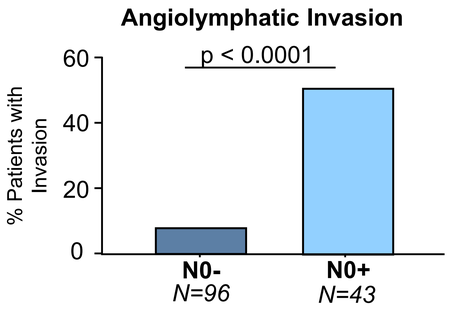


Figure 2. Comparison of angiolymphatic invasion between N0- and N0+ patients. The clinical significance suggests that the genes that control the vascularity of tumors may be the strongest candidates for biomarker diagnosis in primary HNSCC tumors.

CBX5 is Downregulated in Aggressive HNSCC

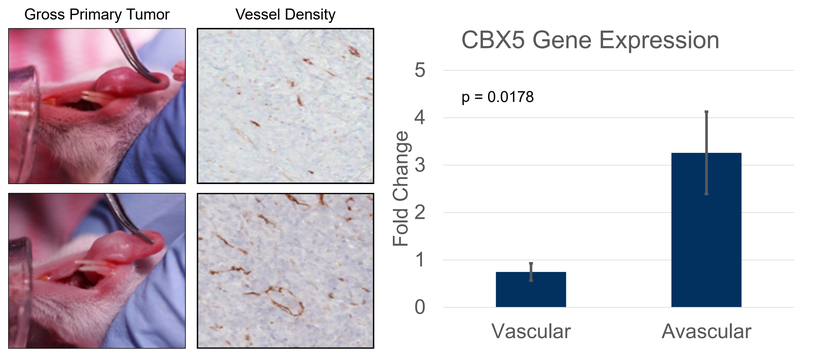


Figure 3. CD31 staining of blood vessels of HNSCC primary tumors in mice, which helped determine which HNSCC metastatic cell lines were vascular and avascular.

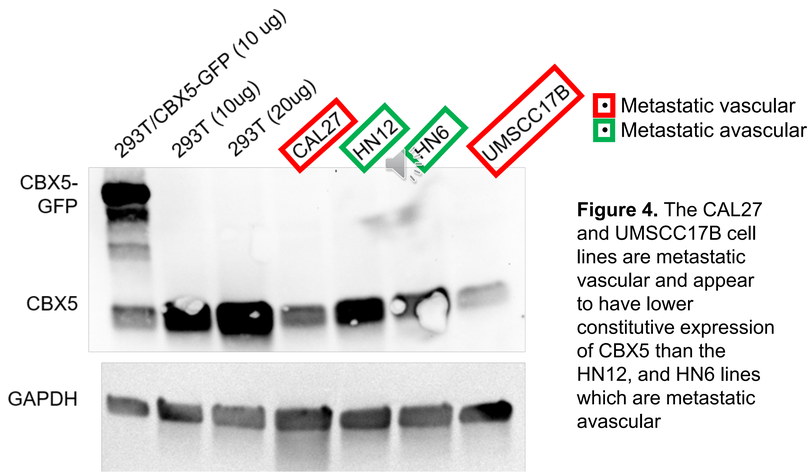
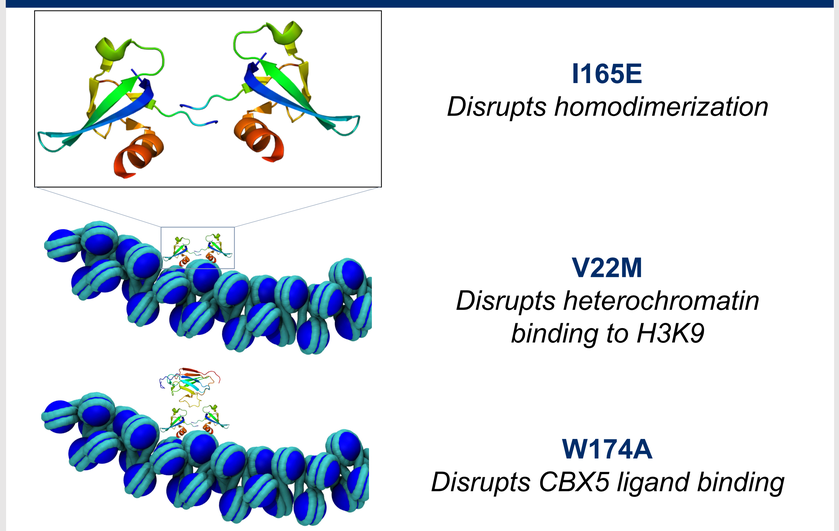


Figure 4. The CAL27 and UMSCC17B cell lines are metastatic vascular and appear to have lower constitutive expression of CBX5 than the HN12, and HN6 lines which are metastatic avascular

Dissecting CBX5 Function Through Mutations



I165E
Disrupts homodimerization

V22M
Disrupts heterochromatin binding to H3K9

W174A
Disrupts CBX5 ligand binding

Introduction of CBX5 Point Mutations

Figure 5. Induction of the V22M point mutation interrupts CBX5's ability to recognize the heterochromatin and bind to it. V22M-1 also had an additional unplanned mutation resulting in a frameshift and resultant total knockdown of CBX5 expression which fortuitously can be used as a negative control.

CBX5 Gateway Cloning

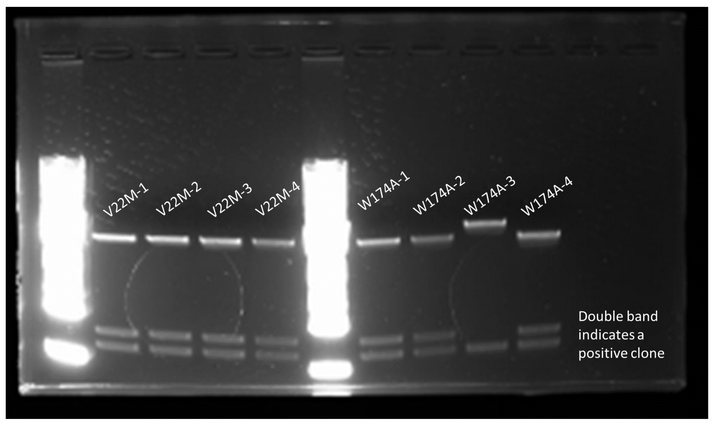


Figure 6. After early restriction digests showed a failed attempt to introduce I165E mutations we proceeded to only clone our V22M and W174A mutants to send for sequencing. In a preliminary restriction digest all 4 V22M mutants had correct lengths, and three W174A mutants were the correct length. These 7 were then sent for genetic sequencing to verify the correct mutations were present

Acknowledgements

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