Title: Oncogenic hub genes ANLN and CTHRC1: Implications for cancer prognosis and vaccine-based therapeutics

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Abastact: This study explores the transcriptomic, mutational, and immunogenic characteristics linked to significantly differentially expressed genes (DEGs) in colorectal (COAD), liver (LIHC), lung (LUAD), gastric (STAD), and breast (BRCA) cancers. Applying integrated bioinformatics algorithms, we discovered common upregulated and downregulated hub genes and assessed their prognostic importance, genomic modifications, copy number variations, functional enrichment, and pathway engagement. The persistent overexpression of ANLN and CTHRC1 in five cancer types, along with poor survival outcomes, underscores their suitability for multi-epitope vaccine development, emphasizing their antigenic potential and significance as universal therapeutic targets. Five genes—ABCA8, PDK4, MT1M, TMEM100, and LIFR—exhibited consistent downregulation and demonstrated tumor-suppressive characteristics. Genomic analyses demonstrated elevated mutation frequencies in ABCA8 and LIFR, predominantly C>T transitions that suggest age-related mutational signatures. Copy number alterations confirmed oncogenic amplifications (ANLN and CTHRC1) and tumor suppressor deletions (e.g., ABCA8). Functional enrichment associated differentially expressed genes with mitosis, chromosome segregation, and metabolic pathways. A multi-epitope vaccine targeting ANLN and CTHRC1 has been established leveraging predicted B-cell and T-cell epitopes, β-defensin as an adjuvant, and efficient linkers. Structural validation indicated desirable folding, stability, and solubility. The vaccine exhibited significant MHC binding, accomplishing 99% global population coverage, alongside strong immune simulation findings. Codon optimization and subsequent cloning into the pET28a(+) vector confirmed the preparation for bacterial expression. ANLN and CTHRC1 demonstrate significant targets for universal immunotherapy. The multi-epitope vaccine demonstrates significant efficacy *in silico* and has the potential to be widely employed as a cancer immunotherapeutic.

Keywords: Pan-cancer analysis Differentially expressed genes ANLN CTHRC1 Cancer immunotherapy Genomic alterations Prognostic biomarkers *In silico* vaccine design

10.1016/j.compbiolchem.2026.109060

https://www.sciencedirect.com/science/article/abs/pii/S1476927126001854