

**Short Communication**

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# Recurrent COQ8B-NUMBL Fusions are the Result of Transcriptional Readthrough Events Frequently Found in Solid Tumors

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## Abstract

Gene fusions are vital in oncogenesis, although they are rare and represent the sole drivers in a minor fraction of cancers. These fusions often stem from genomic rearrangements, notably translocations, which either enhance oncogenic functions or diminish tumor suppressor gene expression. Their detection and interpretation is important for diagnostic and therapeutic stratification efforts. In this context, the proposed ADCK4(COQ8B)-NUMBL gene fusion has been suggested as a frequent event driving cutaneous squamous cell carcinoma (cSCC) development. However, our analysis contests this assertion, demonstrating that COQ8B-NUMBL fusions observed in cSCC are not the result of genomic rearrangements but rather arise from transcriptional readthrough events between adjacent genes on chromosome 19. Our study, consisting of RNA sequencing data from a diverse range of solid tumors, challenges the (clinical) relevance of COQ8B-NUMBL fusions. Notably, we observe a substantial prevalence (40.8%) of these fusion transcripts across all tested tumor types. Furthermore, our findings reveal a significant co-occurrence (36.8%) between established oncogenic drivers and the COQ8B-NUMBL transcriptional readthrough event, suggesting this is not a driver in carcinogenesis. These insights highlight the importance of discerning between genuine genomic rearrangements and transcriptional artifacts to refine our understanding of oncogenic mechanisms. Overall, our study underscores the need for cautious interpretation of fusion events and advocates for comprehensive fusion analyses to elucidate their true biological significance in cancer pathogenesis.

**Keywords:** COQ8B-NUMBL; ADCK4; Gene Fusion; Transcriptional Readthrough; Oncogenesis; Solid Tumor

**Abbreviations:** cSCC: cutaneous squamous cell carcinoma

## Introduction

Gene fusions play a critical role in oncogenesis, albeit that in only 1% of cancers, fusions are the sole driver of tumor development [1]. The most common driver fusions stem from a genomic rearrangement or translocation [2-3] and enhance oncogenic function or diminish tumor suppressor gene expression [1]. The detection and interpretation of oncogenic fusions is important for

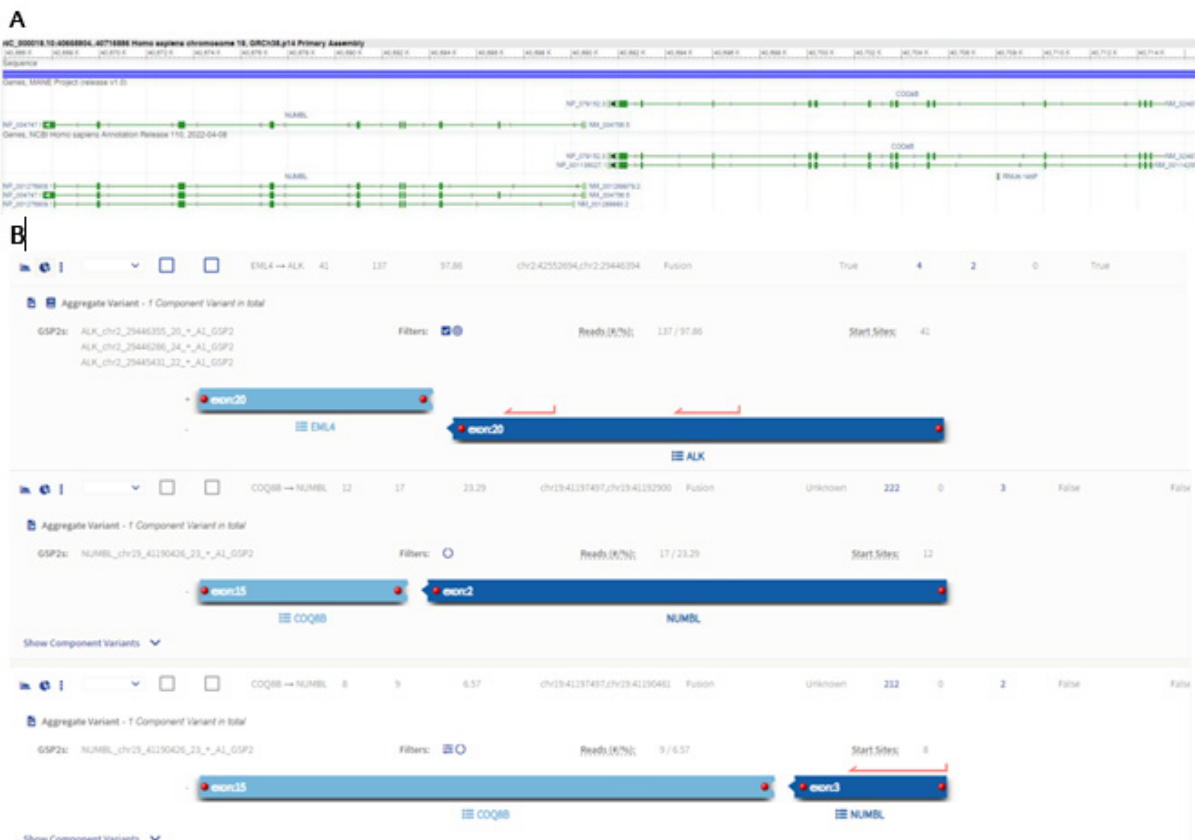
diagnostic and therapeutic stratification. For cutaneous squamous cell carcinomas (cSCC), the gene fusion ADCK4-NUMBL was suggested as a frequent event and driving tumorigenesis [4]. The official symbol for ADCK4 is COQ8B [5] and will be referred to as such. Egashira et al. [4] state that the fusion between COQ8B exon 14, and NUMBL exon 2 is the product of genomic rearrangement of chromosome 19. In this short communication, we contest this

assertion and show that COQ8B-NUMBL fusions are instead the result of a transcriptional readthrough event and frequently found in solid cancer tissues, hence unlikely to be clinically relevant or even specific for cSSC.

**Discussion**

Based on the location within the human genome, the observed COQ8B-NUMBL fusion is not the result of a genomic rearrangement. COQ8B and NUMBL are adjacent genes, located on the complement strand at 19q13.2. As the distance between the two transcripts is only 879 nucleotides (Figure 1A), a transcriptional readthrough is the most likely explanation for the observed transcript, rather than an actual fusion of the two genes. During a transcriptional readthrough, transcription continues into the downstream gene instead of stopping at the canonical termination site, which can also result in alternative splicing variants. Contrarily, readthrough transcripts are observed in a wide range of cases [6] and are associated with cellular stress responses due to a decrease of transcriptional efficiency [6,7]. In order to further support our hypothesis in vivo, we investigated historical data from our routine molecular diagnostics. We recently performed RNA sequencing with the FusionPlex PanSolidTumor v2 18091 v1.0 panel covering NUMBL [8]. This cohort consisted of various

tumors including non- small cell lung carcinomas (n=40), bone and soft tissue tumors (n=18), melanomas (n=9) thyroid tumors (n=4), salivary carcinomas (n=8), diffuse gliomas (n=3), nerve sheath tumors (n=2), hepatic tumor (n=2), metastasis of primary tumors unknown (n=2), prostate carcinoma (n=1), renal carcinoma (n=1), endometrial carcinoma (n=1), myeloid neoplasm (n=1) and a glomus tumor (n=1). Our data shows that COQ8B-NUMBL fusion transcripts (Figure 1B) are not unique for cSSCs and can be found in a variety of solid tumors, such as non-small cell lung carcinomas, melanomas, and salivary carcinomas. In 93 cases recently sequenced, 38 (40.8%) of the samples presented with COQ8B-NUMBL fusion transcripts. Interestingly, this frequency is close to the frequency of COQ8B-NUMBL fusions found by Egashira et al. (i.e. 35.3%) [4]. Since Egashira et al. used a different NGS strategy, it is likely that this frequency is indicative of the same readthrough artifacts and not an artifact specific to our panel. Fourteen (15.1%) of the analyzed tumor tissues contain multiple transcript fusions of COQ8B-NUMBL, indicative for a variety of splice variants of the readthrough transcript. While we detect transcripts involving exon 14 of COQ8B and exon 2 of NUMBL as reported by Egashira et al., in 92.1% of cases other fusion transcripts were detected. In fact, the most commonly found fusion transcript (71.1%) is the COQ8B exon 15:: NUMBL exon 3 fusion transcript.



**Figure A:** Overview of human NUMBL-sapiens chromosome 19 (GRCh38 readthrough.p14 Primary event and Assembly), notagenu position neon-cogenic 40,665;905:40,716,886, gene fusion complement strand. Both the NUMBL and COQ8B transcripts are shown. The first track represents the annotation by the MANE Project (release v1.0); the second tracks represents the NCBI Homo sapiens Annotating Release 110.

Importantly, our findings reveal a significant co-occurrence (36.8) between COQ8B- NUMBL fusions and established oncogenic drivers, both actual fusions and variants (Table 1), like CRTCL1-MAML2 fusions in salivary carcinoma (specifically mucoepidermoid

carcinomas), EML4-ALK fusions in non-small cell lung carcinomas and NAB2-STAT6 fusions in solitary fibrous tumors. This finding supports our hypothesis that COQ8B-NUMBL fusion transcripts do not act as drivers in carcinogenesis.

**Table 1:** analyses–TumorwithsampletheFusionPlexwithCOQ8BPanSolid Tumor NUMBLtranscripts v218091 and v1.0 estabpanelished [8] were fused and collected between November 28, 2022 and January 9, 2023. Each sample was analyzed with Archer Analysis software 6.2.3 and the number of COQ8B- NUMBL fusions, other fusions and variants was recorded. Low confidence fusions and known artifacts were excluded from analysis. Tumor type was taken from the associated patient history and classified with the help of Oncotree [10].

	COQ8B-NUMBL			Other fusions	Variants	Tumor type
13		exon 15::exon 2	exon 15::exon 3		EGFR p.(E746_A750del)	Non-Small Cell Lung Cancer
45			exon 15::exon 3		EGFR p.(E746_A750del)	Non-Small Cell Lung Cancer
14		exon 15::exon 2		CD74(exon 6)::ROS1(exon 34)		Non-Small Cell Lung Cancer
44		exon 15::exon 2		COL1A1(exon 40)::PDGFB(exon 2)		Melanoma
4	exon 14::exon 2		exon 15::exon 3	CSF1(exon 8)::chr1 intergenic		Tenosynovial Giant Cell Tumor Diffuse Type
87		exon 15::exon 2		CTNNB1(exon 1)::PLAG1(exon 3)		Salivary Carcinoma
34	exon 14::exon 2	exon 15::exon 2		EML4(exon 13)::ALK(exon 20)		Non-Small Cell Lung Cancer
12		exon 15::exon 2	exon 15::exon 3	EML4(exon 20)::ALK(exon 20)		Non-Small Cell Lung Cancer
3			exon 15::exon 3	FUS(exon 6)::CREB3L2(exon 5)		Low-Grade Fibromyxoid Sarcoma
56			exon 15::exon 3	FUS(exon 7)::CREB3L2(exon 5)		Low-Grade Fibromyxoid Sarcoma
62			exon 15::exon 3	FUS(exon 7)::DDIT3(exon 2)		Liposarcoma
84			exon 15::exon 3	NCALD(exon 1)::PLAG1(exon 3)		Salivary Carcinoma
8			exon 15::exon 3	TMPRSS2(exon 1)::ERG(exon 4)		Prostate Adenocarcinoma
39			exon 15::exon 3	ZCCHC8(exon 2)::ROS1(exon 36)		Melanoma

## Conclusion

In conclusion, we show that the COQ8B-NUMBL fusions do not result from genomic rearrangements, but from a transcriptional readthrough event of two adjacent genes. This event occurs in 40.8% (38/93) recently analyzed solid tumors, independent of tumor type, and is regularly found (36.8%) in combination with known oncogenic drivers. These insights highlight the importance of discerning between genomic rearrangements and transcriptional artifacts to refine our understanding of oncogenic mechanisms. (2022-04-08) and includes different transcript variants. Figure was generated by NCBI Sequence Viewer 3.47.0 [9]. B. Archer output of RNA sequencing of sample 12, two COQ8B-NUMBL transcripts co-occurred with the established EML4-ALK fusion in a non-small cell lung carcinoma.

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## Conflict of Interest

The authors state no conflict of interest.

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