Profiling of *NUMTs* in Gingivobuccal Oral Cancer

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Abstract

Very little information exists on the Nuclear Mitochondrial sequences (*NUMTs*) in oral squamous cell carcinoma-gingivobuccal (OSCC-GB). We analysed whole genome sequences obtained from paired tumour and blood DNA from 25 patients of OSCC-GB for detecting *NUMTs*. Nine potential somatic *NUMTs* and 78 germline *NUMTs* were identified in these patients. None of the somatic *NUMTs* could be confirmed by PCR assay. None of the germline *NUMTs* were found to be specific for OSCC-GB. Although there have been recent reports on detection of somatic *NUMTs* in other cancer types, our results suggest *NUMTs*, both somatic and germline, are not involved in OSCC-GB.

Introduction

The mtDNA fragments localized into the nuclear genome are identified as Nuclear Mitochondrial sequences (*NUMTs*) and the processes which generate *NUMTs* are collectively known as *NUMTogenesis* (Singh et al., 2017). Irrespective of the insertion site and the mtDNA sequence, all *NUMTs* found in human are rendered non-coding. This pseudogenization of *NUMTs* is due to the difference in codon usage between the nDNA and the mtDNA (Gellissen et al., 1987; Perna et al., 1996).

Although role of *NUMTs* in mitochondrial-nuclear genome evolution has been studied considerably, information on the *de-novo NUMTs* in both sporadic (Turner et al., 2003) and inherited diseases (Goldin et al., 2004) are scarce. Involvement of *NUMTs* in cancer remains a relatively underexplored yet important area of research.

Study participants and methods

Twenty-five patients of gingivobuccal oral cancer (OSCC-GB) were recruited with voluntary informed consent (ICGC India Project Team, 2013). Blood samples collected with informed consent from seven non-cancer individuals were used as control samples.

Whole genome sequence data was generated from paired tumor and blood DNA of 25 OSCC-GB patients by paired end sequencing using Illumina HiSeq-2500. BWA-MEM (Li, 2013) was used to align the whole genome FASTQ files to the human reference sequence (hg19) + mitochondrial reference sequence (rCRS- revised Cambridge Reference Sequence). Alignment files thus produced were used as input for Discovery of Nuclear Mitochondrial Insertions

(DINUMT; Dayama et al., 2014) to identify *NUMTs*. Circos plots shown in this study were generated using BioCircos (Cui et al., 2016).

For the PCR validation of the *NUMTs*, the individual primer pairs were designed in a manner, such that they would amplify the entire length of the *NUMT* + at least 150 bases upstream + at least 150 bases downstream of the insertion site and in absence of *NUMTs* they will amplify only the buffer region (**Supplementary Table S1, Supplementary Table S2**).

Results

NUMTs detected in both paired tumours-blood samples were defined as germline *NUMT* (*G-NUMT*) and *NUMTs* present only in tumours samples but not in paired blood DNA were defined as somatic *NUMT* (*S-NUMTs*). Using *DINUMT*, we could detect 50 such *S-NUMTs* in 25 OSCC-GB patients. Each of these *S-NUMTs* were visually inspected using IGV (Robinson et al, 2011). After verification in IGV, we found 9 potential *S-NUMTs* in 6 patients were supported by at least two discordant reads and one split read (**Figure 1A, Table 1**). Only these 9 *S-NUMTs* which were supported by both discordant and split reads were investigated further.

Figure 1: (A) Circos plot representing insertion location of *S-NUMTs* across chromosomes as identified by *DINUMT*. (B) Circos plot representing insertion location of *G-NUMTs* across chromosomes as identified by *DINUMT*.

Chr. No.	Insertion Start	Insertion Stop	NUMT length	Patient No.
20	45709144	45709146	3895	119
1	210731831	210731888	6770	128
11	17491095	17491097	5087	129A*
17	77335608	77335610	4304	129B*
X	22480071	22480095	1933	129C*
4	47023035	47023211	5436	131A*
X	144335757	144335899	559	131B*
7	140921797	140921880	5958	135
12	94944428	94944538	585	136

Table 1: List of somatic mutations identified by DINUMT with their chromosomal location, length of the mitochondrial insert and patient no. *NUMTs were designated alphabetically when a single patient harbour more than one S-NUMTs.

A total of 78 G-NUMTs were identified in 25 patients (**Figure 1B, Table 2**). We named the G-NUMTs as follows: *chr-NO.-occurrence*. Five recurrently reported G-NUMTs present in chromosome 11 (2nos; chr-11-15, chr-11-11) chromosome 8 (chr-8-5), chromosome 4 (chr-4-11) and chromosome 2 (chr-2-4) were selected for confirmatory assay. Additionally, no previously reported NUMTs were found in the neighbouring genomic region (1kb upstream to 1kb downstream of the predicted insertion site) from each of the identified G-NUMT sites by UCSC genome browser with the NUMT track.

Chr. No.	Insertion Start	Insertion Stop	NUMT Length [#]	Frequency	Patient No.														
11	4656342	4656344	44	15	113	116	119	120	121	122	125	126	129	130	133	134	138	139	140
4	29441246	29441248	1353	11	116	117	119	120	121	122	125	126	137	139	140				
11	49883568	49883572	377	11	113	117	119	122	130	133	134	135	137	138	140				
11	77213043	77213050	39	8	113	120	121	122	125	126	138	139							
8	63015422	63015431	121	5	116	119	122	129	133										
2	33892478	33892482	130	4	121	134	137	140											
2	214283781	214283783	37	4	119	134	139	140											
3	56147220	56147222	63	3	117	119	139												
5	8255946	8255948	43	3	116	119	138												
5	32338582	32338584	2198	3	116	130	138												
21	23080617	23080619	*	3	113	122	140												
1	59661551	59661553	18	2	113	133													
4	83616162	83616164	*	2	120	121													
11	100015736	100015750	60	2	126	133													
13	64162211	64162216	*	2	133	134													

Table 2: List of germline mutations identified by DINUMT with their chromosomal location, length of the mitochondrial insert, frequency in the patients and the patients harbouring those NUMTs. [#] length of the longest NUMT observed in any of the patient carrying that NUMT is reported. *length of the NUMT could not be determined by DINUMT.

In 4 (119, 129C, 131B, 136) out of the 9 potential S-NUMTs, PCR-amplicons of length spanning the respective S-NUMT and the buffer region was observed from both tumour and matched blood. In two potential S-NUMTs (128, 131A), amplification of only buffer region was observed from both tumours and blood. No amplicons were observed for rest of the three potential S-NUMTs (129A, 129B, 135).

G-*NUMT* chr2-4 was detected in all 4 patients by PCR. G-*NUMT* chr8-5 was found to be present in blood and tumour DNA in only one patient. Presence of G-*NUMT* chr4-11 was detected in 3 OSCC-GB patients. Only three patients carried G-*NUMT* chr11-11. G-*NUMT* chr11-15 was detected in all 15 patients by PCR. Seven control individuals investigated from G-*NUMT* chr11-15 by PCR were positive for the specific amplicon. None of the G-*NUMTs* identified here interfered with ORF of any nuclear genes.

Discussion

In 25 OSCC-GB patients investigated here, none harbour somatic *NUMTs*. Out of the 9 potential S-*NUMT* candidates identified by computational approach, none of them could be verified by PCR as somatic events. We have identified a number of germline *NUMTs* in oral cancer patients. But our study lacks the sample size required for estimating association of the observed G-*NUMTs* with oral cancer. The only G-*NUMT* recurrently observed in our sample set (chr-11-15) was also found to be present in blood DNA derived from non-cancer individual.

In a recent study, *Ju et al., 2015* have observed S-*NUMTs* in 12 breast cancer samples. Among these, only 10 are primary patient samples out of a total of 559 samples harbouring various types of tumours (ALL, AML, breast cancer, lung cancer, colorectal cancer, RCC, melanoma, glioma, prostate cancer etc.) studied in the TCGA project. These events could not be correlated with any clinical phenotype of cancer and were considered as passenger events. Our result, combined with the reports previously mentioned, indicates that S-*NUMTs* are ambiguous in nature and probably they occur in certain specific cancers (*Ju et al., 2015; Yuan et al., 2017*) and not others e.g. gingivobuccal oral squamous cell carcinoma.

To conclude, we did not find any OSCC-GB specific *NUMT*. Whether any or all of the G-*NUMTs* detected by us are novel polymorphic *NUMTs* remain to be verified in large data set. Our study of germline *NUMTs* in these patients did not reveal any evidence for association with OSCC-GB.

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Declaration of interest

The authors declare no conflict of interest.

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