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Sequencing of within-Subject Primary and Metastatic Cutaneous Squamous Cell Carcinomas Reveals Novel Diagnostic and Therapeutic Targets for Metastatic Disease

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Abstract

Cutaneous squamous cell carcinoma (cSCC) is a common type of skin cancer that rarely metastasizes. However, metastasis is associated with significant morbidity and mortality. Thus, additional study on the molecular markers that prognosticate aggressive behavior of cSCC are needed to improve diagnostic and therapeutic tools. Our goal was to identify genetic mutations in cSCCs that may be markers of or contribute to metastatic risk. A matched case-control study of high depth sequencing of 397 genes in matched metastatic and primary cSCC from 12 patients was performed in 2020. A follow up of at least 5 years was required for all patients. Identified mutations were compared between the two groups. Over 1,000 deleterious mutations were identified in metastatic and primary cSCC samples, of which TP53, LRP1B and FAT1 mutations were most common overall. In comparing between the metastatic and primary cSCCs, 53 genes were unique to the metastatic group. Mutations in SETBP1, AR, and TSHR were most common and unique to the metastatic samples. Though also found in primary cSCC, NOTCH1 was significantly more commonly mutated in metastatic samples than primary cSCC.We nominate several genes that, when mutated, serve as markers for more aggressive behavior in cSCCs. Further investigations into this complex but important topic are needed.

Introduction

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer in the US. Although metastasis of cSCC to lymph nodes occurs in only 2-4% of cases in most individuals [1], in severely immunocompromised subjects nodal metastasis can occur in ~20% of cases [2,3]. Once cSCC has metastasized, mortality rates are comparable to melanoma, renal cell carcinoma and oropharyngeal carcinoma [4], with a survival rate of 25-35% at 5 years and <10% at 10 years [5]. As a result, there is a pressing need to understand the molecular events that drive cSCC towards metastasis.

Clinical and histopathologic characteristics can help predict the risk for cSCC metastasis [6]. Anatomic location on the ear/lip increases metastasis risk to 11-13%; development of cSCC from a scar has a risk of ~40% [4]. From a histologic standpoint, poorly differentiated tumors (32.8%), tumors >2 cm in size (~30%), previously treated tumors (~30%), and perineural invasion (47.3%) also confer high metastasis risk [4]. Staging criteria from the American Joint Committee on Cancer and the National Comprehensive Cancer Center Network have been put forth to help physicians distinguish high risk from low risk cSCCs, but agreement between criteria by different organizations is poor [7].

In other cancers, profiling of signature genetic alternations can be used to predict tumor behavior and therapeutic response. However, mutational signatures that predict metastasis risk in cutaneous SCC have not been well defined. In oral SCC, mutations in TP53, CASP8, BRCA2 and FAT1 have been identified in metastatic vs localized cancers[8]. A four gene expression signature of EGFR, HER-2/neu, LAMC2 and RHOC had a specificity of 87.5% and a sensitivity of 70% with a prognostic accuracy of 83.4% for lymph node metastasis in oral SCC [9]. Whole-exome sequencing of metastatic head and neck SCC found enriched mutations in inositol 1,4,5-triphosphate receptor type 3 (ITPR3), C17orf104, and discoidin domain receptor tyrosine kinase 2 [10]. However, the genetic underpinnings of SCC from oral and head/neck sites are known to be distinct from cutaneous SCC [11].

Studies of primary cutaneous SCC identified p53 as the most frequently inactivated tumor suppressor protein [11], with additional recurrently mutated genes including retinoblastoma, cyclin D1, cyclin-dependent kinase inhibitors (ink4 family (p16), Cip/Kip family (CDKN1A), and p27 (CDKN1B). Other studies found additional mutations in KMT2D, AFF3, ROS1, TERT, CACNA1D, KMT2A, PED4DIP, GRIN2A, SPEN, KMT2C, ZNF521, TRRAP, KDR, CREBBP, EP300, SMARCA4, and RANBP17 [12]. Involvement of HPV, particularly the beta type, has also been implicated[11]. Despite these invaluable mutational data, further knowledge on the drivers of metastatic disease could help in the development of diagnostic and therapeutic tools. One critical lesson learned from studies of head and neck SCCis that synchronous lymph node metastatic tumorswere genetically more similar to index primary tumors than to metachronous recurrent tumors. This indicates that defining signature mutations for metastatic cSCC should rely on paired primary and metastatic tumors collected from the same patient.

More recently, Lobl et al. (2020) [13] investigated the differences in 76 genes in 20 case-matched localized and metastatic cSCCs. EGFR mutations were found to be the primary driver mutations whereas CDH1 was found to be the primary driver mutation in

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metastatic SCC. RTK/RAS, TP53, TGF-B, NOTCH1, PI3K and cell cycle pathways were found to contribute to high risk SCCs. The Wnt pathway was enhanced in metastatic SCC only. These results form a basis for further investigation into the complex genetics of metastatic cSCC.

Here, we performed high-depth sequencing of a 397 cancer gene panel in a cohort of patient-matched primary and metastatic cSCC from 12 patients. Our results describe the landscape of genes and mutations associated with cSCC metastasis.

Methods

Cutaneous SCCs treated with Mohs micrographic surgery at the UC San Diego Dermatology department between January 2007 and December 2012 were included in the study. Internal review board approval was obtained. Cases were included if at least 5 year clinical follow-up was available. We identified patients with both local/primary cSCCs and metastatic cSCCs. Local/primary lesions were defined as biopsy-proven cSCCs with no clinical evidence of metastasis at 5 years. Metastatic lesions were defined as cSCCs clinically thought to have metastasized and beresponsible for metastatic disease documented by imaging or biopsy (and not the actual metastatic lesion, which may be lymph node, lung, or organ other than skin). For optimal matching of cases, patients with both local/primary lesions and metastatic lesions were identified and their matching tissue samples were sequenced. Demographic information, clinical characteristics, and surgical characteristics were collected. Archived formalin-fixed samples were identified and the histopathology was reviewed to identify sections containing tumor tissue and, when available, patient-matched normal skin controls. Genomic DNA was isolated from paraffin sections of the metastatic, primary, and normal tissues using a QIAmp DNA FFPE tissue kit (Qiagen). Twenty-eight samples from 12 patients were included in the study.

Clinical Sequencing

Sequencing was carried out using a custom SureSelect hybridization-based capture (Agilent) and KAPA Hyper Prep library preparation kits (Roche) using 100ng input genomic DNA. The hybridization-based capture probes target the coding regions (exons) for a panel of 397 genes (Supplemental Table 1). Enriched genomic regions were sequencedon a HiSeq2500 sequencing instrument (Illumina) and yielded 600X average depth of coverage.

Bioinformatic Analysis

Paired end read files (fastq) are aligned to the Genome Reference Consortium Human Build 37(GRCh37) reference sequence using BWA-MEM [14], local realignments were carried out using GATK [15], and variant calling was carried out using Lofreq [16] to identify single nucleotide variants and small insertions and deletions that are represented by aminimum of 100 reads. Copy number abnormalities were determined by comparison of sequence coverage of targeted regions in the tumor sample relative to a set of standard diploid samples. Log-ratio values were calculated and segmented using circular binary segmentation [17]. Tumor mutation burden (TMB) was calculated as the total number of mutations observed/megabase (Mb) ofDNA sequenced [18].

Threshold quality metrics for each sample included average 300X depth of coverage, 95% of all bases in targeted regions represented by at least 100 reads, and minimum threshold of 15 million total mapped reads for each sample. Variants were defined as those with > 5% variant allele frequency with >100 raw variant reads.

Variant Interpretation

Annotation of variants was performed by ANNOVAR [19] and Variant Effect Predictor [20]. Coding variants, variants within 5 bases of a coding region, or previously characterized intronic variants were carried forward. Potential deleteriousness was assessed by SIFT [21,22], Polyphen-2 [23], and Mutation assessor [24,25]. In addition, all variants were assessed to determine if they may affect splicing using Variant EffectPredictor [20]. Each variant was compared to ClinVar [26] to determine if the variant has been previously reported, and the reported classification. Population frequencies were also determined for each variant using the Genome Aggregation Database (gnomAD) and 1000 Genomes database.

Variants with a population frequency of < 1% in either database were carried forward. All variants were interpreted by a clinicalmolecular geneticist (SM) for a final classification as clinically significant, likely clinically significant, a variant ofunknown significance, or a not reportable variant (likely benign/benign).

Statistical Analysis

Statistical analyses were performed using SPSS 20 statistical software (SPSS 20.0, SPSS Inc. Chicago, IL, USA). The Related-Samples Wilcoxon Signed Rank test was used to compare if there was a difference between primary tumor mutation load and metastatic tumor mutation load. Statistical tests were two-sided and a p-value <0.05 was considered statistically significant.

Results

Twelve patients with both metastatic and primary cSCC were identified. From each case, tissue from both metastatic and primary tumors were sequenced. Four cases also had available normal tissue controls, which were sequenced. Table 1 displays the clinical characteristics of the cases.

A total of 2362 protein-coding mutations were identified in a panel of 397 cancer-associated genes. Filtering by SIFT identified 1082 mutations as likely to be damaging or deleterious. Of these mutations (Table 2), the average number of mutations in metastatic samples across the dataset was 48.4 (20.9 mutations per megabase), compared with 37.9 mutations in primary cSCC samples (18.4 mutations per megabase). For comparison, 11.5 mutations were found per sample in the subset of normal non-cSCC samples (7 mutations per megabase).

A total of 581 mutations were identified in metastatic cSCC samples, with some samples displaying multiple deleterious mutations in the same gene. Overall, TP53 mutations were most common (18 mutations), followed by LRP1B (17), FAT1 (16) and NOTCH1 (16). A total of 455 mutations were identified in primary cSCC samples. FAT1 mutations were the most common (14), followed by LRP1B (11), TP53 (10) and SPTA1 (10). Within the normal samples, there was no significant enrichment for mutations in any genes.

We compared detected mutation frequencies against previous mutational studies of cSCC (Figure 1). Our results were largely in accordance with prior reports, with top mutated genes identified in cBioportal [27] (TP53, LRP1B, NOTCH1 and ROS1) also highly represented in our dataset.

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Table 1. Clinical characteristics and demographics of 12 cases included in the study.

Study ID	Age	Sex	Cause of Immunosuppression	Location of Primary Lesion	Location of Metastasis
Patient 1	62	M	None	Right neck	Bone, Lymph node
Patient 2	55	F	None	Left wrist	Lymph node L axilla
Patient 3	78	M	Chronic lymphocytic leukemia	Scalp	Lymph node and in transit
Patient 4	69	M	Human immunodeficiency virus	Left face	Lymph node
Patient 5	86	M	Myelofibrosis	Occipital scalp	In transit
Patient 6	66	M	Amyloidosis	Scalp	In transit
Patient 7	70	M	Chronic kidney disease	Right neck	Parotid and Lymph node
Patient 8	69	M	Lung transplant	Scalp	Lung on autopsy, Lymph node, and in transit
Patient 9	96	F	None	Right cheek	Lymph node
Patient 10	96	F	None	Scalp	In transit
Patient 11	70	M	Lung and kidney transplant	Posterior scalp	In transit
Patient 12	73	M	Lung transplant and myelodysplastic syndrome	Left temple	Parotid

Table 2. Table detailing the percentage of mutations and mutations per megabase in primary, metastatic, and normal samples. Mut/Mb = Mutations per megabase.

Study ID	Total mutations Metastatic	Total mutations Primary	Total mutations Normal	Mutations /Mb Metastatic	Mut/Mb Primary	Mut/Mb Normal
Patient 1	10.3%	9.8%	NA	4.6%	4.6%	NA
Patient 2	6.8%	8.6%	1.5%	2.8%	4.6%	1.2%
Patient 3	1.8%	16.6%	NA	0.9%	8.4%	NA
Patient 4	34.0%	11.1%	NA	16.3%	4.0%	NA
Patient 5	12.6%	8.6%	NA	6.5%	4.6%	NA
Patient 6	11.1%	11.3%	NA	4.9%	4.5%	NA
Patient 7	3.5%	4.0%	3.8%	2.0%	1.8%	2.2%
Patient 8	6.8%	11.8%	4.8%	3.7%	6.6%	2.4%
Patient 9	20.4%	5.5%	1.5%	4.6%	3.3%	1.3%
Patient 10	6.3%	6.5%	NA	3.2%	2.9%	NA
Patient 11	15.4%	4.0%	NA	5.3%	2.2%	NA
Patient 12	17.4%	16.6%	NA	8.3%	8.2%	NA
Average	12.2%	9.6%	2.9%	5.3%	4.6%	1.8%

We next sought to compare the gene mutational landscape between primary vs. metastatic cSCC tumors. There were 136 mutated genes shared between metastatic and primary groups, with 53 genes mutated uniquely in the metastatic samples. The most common differentially mutated genes are listed in Table 3.

NOTCH1 and LRP1B mutations appear to be more commonly mutated in metastatic samples compared with primary samples. In contrast, SPTA1 and IKBKE appear to be more commonly mutated in primary samples compared with metastatic samples. The most commonly mutated genes in metastatic samples that were not mutated in primary samples include SETBP1, AR, and TSHR.

Though the power of our study is limited by small sample size, we used the Wilcoxon signed rank test to globally compare the number of mutations in the primary and metastatic cSCC samples and sought to identify differences in mutation rates of each gene. We found that metastatic tumors had a significant increase in mutations (mean, 3.73 ± 2.94 vs 1.80 ± 2.58; median, 3.00 vs 1.00; $p<0.001$). Among individual genes, NOTCH1 wassignificantly more commonly mutated in metastatic than primary cSCC.

Discussion

In this study, we performed high-depth analysis of 397 genes to compare the mutational landscapes of 12 patient-matched metastatic and primary cSCCs. We found that in general, metastatic cSCCs had greater mutational burden compared with patient-matched primary cSCCs. Our dataset agreed with the prior literature in the genes most commonly mutated in cSCC. In our cohort,we noted that NOTCH1 and LRBP1 mutations were more common in metastatic cSCC than primary cSCC. We also observed several genes that were uniquely mutated in metastatic samples that were not mutated in matched primary tumors, which included SETBP1, AR, and TSHR.

Our results agreed and contrasted with those of Lobl et al.[13], which highlights the challenges of identifying consistent mutation signatures in this highly variable cancer and may reflect the mutational heterogeneity in metastatic cSCC. Both studies identified NOTCH1 as being associated with high-risk SCCs. In our study, CDH1 was found to be mutated in metastatic tumors and not primary tumors in a subset of samples, whereas Lobl et al. found CDH1 to be a primary driver mutation. Further, the WNT pathway was found to be important in metastatic cSCCs in the Lobl article; similarly, our study identified WNT cascade-members LRP and AXIN1 mutations to be enriched in metastatic compared with primary samples. RTK/RAS, TGF-b and PI3K pathways were not associated with metastatic cSCCs in our study. Cell cycle, growth, and proliferation pathways in both datasets appear to impact the propensity of cSCCs to metastasize.

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Table 3. Frequency of mutations found in genes with greatest differences between metastatic and nonmetastatic samples. The number of mutations per gene per sample and total mutations in that gene are listed. Patient-matched normal skin controls did not have any mutation for all of the genes.

Gene	Metastatic cSCC (n=12)		Primary cSCC (n=12)	
	Frequency	Total Mutations	Frequency	Total Mutations
LRP1B	9 (75.0%)	17	8 (66.7%)	11
NOTCH1	8 (66.7%)	16	3 (25.0%)	4
NOTCH2	6 (50.0%)	8	4 (33.3%)	5
TRRAP	6 (50.0%)	7	3 (25.0%)	3
SPEN	6 (50.0%)	7	2 (16.7%)	2
KAT6B	5 (41.7%)	6	1 (8.3%)	1
TSC2	4 (33.3%)	6	3 (25.0%)	3
EP300	4 (33.3%)	5	1 (8.3%)	1
CLTCL1	4 (33.3%)	5	2 (16.7%)	2
CACNA1D	4 (33.3%)	4	1 (8.3%)	1
PTPRC	3 (25.0%)	5	1 (8.3%)	1
MED12	3 (25.0%)	4	1 (8.3%)	1
IRS2	3 (25.0%)	4	1 (8.3%)	1
CDKN2A_p16	3 (25.0%)	4	6 (50.0%)	6
BCORL1	3 (25.0%)	4	1 (8.3%)	1
AR	3 (25.0%)	4	0	0
SLIT2	3 (25.0%)	3	2 (16.7%)	5
FOXP1	3 (25.0%)	3	0	0
DICER1	3 (25.0%)	3	1 (8.3%)	1
BRCA2	3 (25.0%)	3	0	0
SETBP1	2 (16.7%)	5	0	0
TSHR	2 (16.7%)	4	0	0
RANBP2	2 (16.7%)	4	4 (33.3%)	6
BCL11B	2 (16.7%)	4	1 (8.3%)	1
STAT5B	2 (16.7%)	3	0	0
STAT3	2 (16.7%)	3	1 (8.3%)	1
PPARG	2 (16.7%)	3	0	0
NUP214	2 (16.7%)	3	1 (8.3%)	1
MAGI2	2 (16.7%)	3	4 (33.3%)	6
KMT2D	2 (16.7%)	3	1 (8.3%)	1
KDM6A	2 (16.7%)	3	1 (8.3%)	1
JAK3	2 (16.7%)	3	1 (8.3%)	1
CYLD	2 (16.7%)	3	0	0
CDK4	2 (16.7%)	3	0	0
BAP1	2 (16.7%)	3	0	0
RAF1	2 (16.7%)	2	0	0
PREX2	2 (16.7%)	2	3 (25.0%)	6
MLH1	2 (16.7%)	2	0	0
LIFR	2 (16.7%)	2	0	0
GPR124	2 (16.7%)	2	4 (33.3%)	5
FANCC	2 (16.7%)	2	0	0
EZH2	2 (16.7%)	2	0	0

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ERBB2	2 (16.7%)	2	0	0
CBLB	2 (16.7%)	2	0	0
CASP8	2 (16.7%)	2	0	0
AXIN1	2 (16.7%)	2	0	0
TRIP11	1 (8.3%)	3	1 (8.3%)	1
TAF1	1 (8.3%)	2	0	0
RARA	1 (8.3%)	2	0	0
PMS1	1 (8.3%)	2	0	0
CDH1	1 (8.3%)	2	0	0
VEGFA	1 (8.3%)	1	2 (16.7%)	3
SPTA1	1 (8.3%)	1	5 (41.7%)	10
IKBKE	1 (8.3%)	1	3 (25.0%)	3
CHEK2	1 (8.3%)	1	2 (16.7%)	3

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Gene	Cbioportal	Normal (n=4)		Primary cSCC (n=12)		Metastatic cSCC (n=12)	
	Frequency	*Frequency*	*Total Mutation*	*Frequency*	*Total Mutation*	*Frequency*	*Total Mutation*
TP53	93 (88.2%)	1 (25%)	1	8 (66.7%)	10	9 (75.0%)	18
LRP1B	127 (70.6%)	0	0	8 (66.7%)	11	9 (75.0%)	17
NOTCH1	69 (54.4%)	0	0	3 (25.0%)	4	8 (66.7%)	16
ROS1	54 (45.6%)	1 (25%)	1	4 (33.3%)	5	7 (58.3%)	9

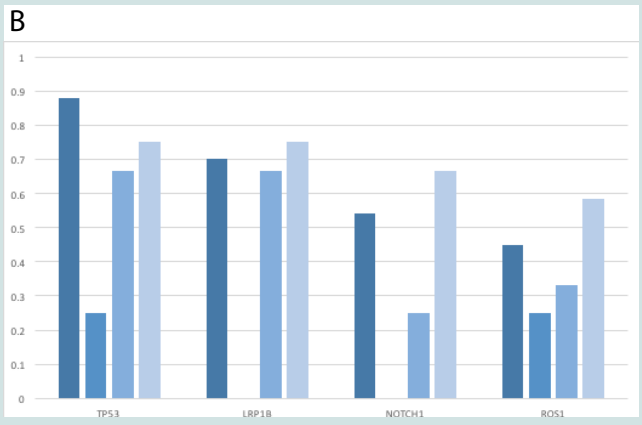

Figure 1: Common cSCC-associated mutations in the dataset. A) Table showing the frequency of common gene mutations in cSCCs based on aggregate data from the literature in CBioportal, compared with frequency of mutations in normal, primary, and metastatic cSCC samples. B) Graphical representation of most commonly mutated genes overall.

Our results indicate that metastatic cSCCs act by different genetic mechanisms compared with mucosal SCCs. Whereas chromosomal instability and DNA repair defects have been associated with mucosal SCCs [8], our dataset suggests that metastasis of cSCCs displays more association with cell growth and proliferation pathways. However, it is interesting that in prior studies of mucosal SCCs and in our dataset, growth and hormone signaling-related molecules such as EGFR and HER2/neu in mucosal SCC, and thyroid stimulating hormone receptor (TSHR) and androgen receptor (AR) in our cSCC dataset, were correlated to metastatic cancers.

The androgen receptor is a ligand-activated nuclear receptor that regulates gene expression in a number of tissues and promotes carcinogenesis in such cancers as prostate cancer and hepatocellular carcinoma [28]. In one study, AR was associated with progression and metastasis in hepatocellular carcinoma, suggesting that tumors not classically linked to sex hormones can still be affected by circulating androgens or alterations in the pathway [28]. Thus, abnormalities in this pathway could also play a role in cSCC metastasis. Interestingly, in our dataset there were both male and female cases linked to the mutation in AR, which suggests that these abnormalities need not be sex-specific.

It is also well known that thyroid hormone can be anti-apoptotic and can support tumor cell proliferation and angiogenesis [29]. Thyroid hormone may act through enhancing the expression of matrix metalloproteinases that aid metastasis by liberating cancer cells from a primary tumor or allowing circulating tumor cells to relocate at a distant site [30]. Further, thyroid hormone affects transcription of angiogenesis-associated VEGF, bFGF, EGFR and PDGF genes [29,31]. In cSCC, thyroid hormone is known to promote a ZEB1/E-cadherin switch that aids in progression and invasiveness of the tumor [32]. In this study, blocking thyroid hormone signaling reduced tumor invasion. Androgens and thyroid hormones have been subject of intense study in other fields as targets for preventing metastasis in different types of cancer, and should perhaps be further investigated in the context of cSCC.

Finally, NOTCH1 is a tumor suppressor gene that has been associated with prostate, pancreas, breast and lung cancers [33]. In oral SCC, NOTCH1 expression is correlated with clinical and T stage, lymph node metastasis and depth of invasion [34]. NOTCH1 mutation in oral SCC can predict worse overall survival and disease free survival in an oral SCC cohort [35]. Our dataset shows that mutations

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in NOTCH1 may also be important in conferring metastatic potential of cSCC as well. However, given the high prevalence of NOTCH1 mutations in cSCC (8/12 in the metastatic cSCC group and 3/12 in primary cSCC group), NOTCH1 may not be helpful in differentiating between cSCCs that will develop the ability to metastasize in a clinical context.

Limitations

Our study had a small sample size and limited power to detect statistical differences in mutational rates of individual genes. Our intent to limit our study to samples from subjects that had both primary and metastatic cSCC samples available and extended (>5 year) documented clinical follow-up resulted in a more restricted cohort, but was designed in an effort to decrease inter-subject mutational variation that could lead to false positive conclusions.

Our cases are from a single tertiary care center in Southern California. The demographic composition of our catchment area is characterized by enrichment for non-Hispanic whites and Hispanic and Latino populations and reduced representation of Black individuals compared to the national average. Our results could be influenced by these and other geographic differences and may not extrapolate to the broader population.

Conclusion

In summary, we present novel gene mutation data from 12 patient-matched primary and metastatic cSCCs using a 397 cancer gene panel. We nominate severalcandidates as potential signature genesthat characterize metastatic cSCCs. Future directions should include larger sample sizes, perhaps through multi-center efforts, to confirm and extend these findings.

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References

1. Thompson AK, Kelley BF, Prokop LJ, Murad MH, Baum CL (2016) Risk Factors for Cutaneous Squamous Cell Carcinoma Recurrence, Metastasis, and Disease-Specific Death. JAMA Dermatology 152:419.
2. Farasat S, Yu SS, Neel VA (2011) A new American Joint Committee on Cancer staging system for cutaneous squamous cell carcinoma: creation and rationale for inclusion of tumor (T) characteristics. J Am Acad Dermatol 64:1051-1059.
3. Alam M, Ratner D (2001) Cutaneous Squamous-Cell Carcinoma. N Engl J Med 344:975-983.
4. Karia PS, Han J, Schmults CD (2013) Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. J Am Acad Dermatol 68:957-966.
5. M, Ratner D (2001) Cutaneous squamous-cell carcinoma. N Engl J Med 344:975-983.
6. Karia PS, Han J, Schmults CD (2013) Cutaneous squamous cell carcinoma: Estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. J Am Acad Dermatol 68:957-966
7. Shulman K, Cohen I, Barnett-Griness O (2011) Clinical implications of UGT1A1*28 genotype testing in colorectal cancer patients. Cancer 117:3156-3162.
8. Biswas NK, Das C, Das S (2019) Lymph node metastasis in oral cancer is strongly associated with chromosomal instability and DNA repair defects. Int J Cancer 2568-2579.
9. Zanaruddin SNS, Saleh A, Yang Y-H (2013) Four-protein signature accurately predicts lymph node metastasis and survival in oral squamous cell carcinoma. Hum Pathol 44:417-426.
10. Hedberg ML, Goh G, Chiosea SI, JE Bauman, ML Freilino, et al. (2015) Genetic landscape of metastatic and recurrent head and neck squamous cell carcinoma. J Clin Invest 126:169-180.
11. Mueller SA, M Emilie, MEA Gauthier, Ashford B, R Gupta, et al. (2019) Mutational Patterns in Metastatic Cutaneous Squamous Cell Carcinoma. J Invest Dermatol 139-1449-1458
12. Ashford BG, Clark J, Gupta R, Iyer NG, Yu B, et al. (2017) Reviewing the genetic alterations in high-risk cutaneous squamous cell carcinoma: A search for prognostic markers and therapeutic targets. Head Neck 39:1462-1469.
13. Lobl M, Clarey D, Higgins S, Sutton A, Hansen L, et al. (2020) Targeted next-generation sequencing of matched localized and metastatic primary high-risk SCCs identifies driver and co-occurring mutations and novel therapeutic targets. J Dermatol Sci 99: 30-41.
14. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25-14.
15. A McKenna, M Hanna, E Banks, A Sivachenko, K Cibulskis et al. (2010) The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20: 1297-1303.
16. Wilm A, Aw PPK, Bertrand D, et al. (2012) LoFreq: A sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Res. 40: 11189-11201.
17. DT Cheng, TN Mitchell, A Zehir, RH Shah, R Benayed, et al. (2015) Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): A hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagnostics 17: 251-264.
18. TR Hodges, Martina Ott, Joanne Xiu, Z Gatalica, J Swensen, et al. (2017) Mutational burden, immune checkpoint expression, and mismatch repair in glioma: Implications for immune checkpoint immunotherapy. Neuro Oncol 19:1047-1057
19. Wang K, Li M, Hakonarson H. (2010) ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:164.
20. W McLaren, L Gil, SE Hunt, HS Riat, GRS Ritchie, et al. (2016) The Ensembl Variant Effect Predictor. Genome Biol 17: 122.
21. Ng PC, Henikoff S (2003) SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 31: 3812-3814.
22. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4: 1073-1081.
23. Adzhubei IA, Schmidt S, Peshkin L (2010) PolyPhen-2 : prediction of functional effects of human nsSNPs. Nat Methods 7(4).
24. Reva B, Antipin Y, Sander C (2007) Determinants of protein function revealed by combinatorial entropy optimization. Genome Biol 8: 232
25. 25. Reva B, Antipin Y, Sander C (2011) Predicting the functional impact of protein mutations: Application to cancer genomics. Nucleic Acids Res 39: 118.
26. MJ Landrum, JM Lee, M Benson, GR Brown, C Chao, et al. (2018) ClinVar: Improving access to variant interpretations and supporting evidence. Nucleic Acids Res 46: 1062-1067.
27. E Cerami, J Gao, U Dogrusoz, BE Gross, SO Sumer, et al. (2012) The cBio Cancer Genomics Portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov 5:401-404.

ISSN: 2373-1044

28. Ao J, J Meng, L Zhu, H Nie, C Yang et al. (2012) Activation of androgen receptor induces ID1 and promotes hepatocellular carcinoma cell migration and invasion. Mol Oncol 6:507-515.

29. SA Mousa, GV Glinsky, HY Lin, OA Fabian, A Hercbergs, et al. (2018) Contributions of thyroid hormone to cancer metastasis. Biomedicines 6: 89.

30. M Hong, H Cheng, L Song, W Wang, Q Wang, et al. (2018) Wogonin suppresses the activity of matrix metalloproteinase-9 and inhibits migration and invasion in human hepatocellular carcinoma. Molecules 23:384.

31. Davis PJ, Sudha T, Lin HY, Mousa SA (2016) Thyroid hormone, hormone analogs, and angiogenesis. Compr Physiol 6: 1.

32. C Miro, ED Cicco, R Ambrosio, G Mancino, DD Girolamo, et al. (2019) Thyroid hormone induces progression and invasiveness of squamous cell carcinomas by promoting a ZEB-1/E-cadherin switch. Nat Commun 10: 5410.

33. Fukusumi T, Califano JA (2018) The NOTCH Pathway in Head and Neck Squamous Cell Carcinoma. J Dent Res 97: 6.

34. R Yoshida, M Nagata, H Nakayama, KN Kita, W Hassan, et al. (2013) The pathological significance of Notch1 in oral squamous cell carcinoma. Lab Investig 93:1068-1081.

35. X Song, R Xia, J Li, Z Long, H Ren, et al. (2014) Common and complex Notch1 mutations in chinese oral squamous cell carcinoma. Clin Cancer Res 20:701-710.