

Genetic Predisposition to Numerous Large Ulcerating Basal Cell Carcinomas and Response to Immune Therapy

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Abstract

Objective: Well-defined germ-line mutations in the *PTCH1* gene are associated with syndromic multiple basal cell carcinomas (BCCs). Here, we used whole exome sequencing (WES) to identify the role of patched-1 in patients with multiple, unusually large BCCs.

Methods: A 72-year old patient presenting with numerous BCCs progressing to large ulcerating lesions was enrolled. WES was used to identify the pathogenic gene locus.

Results: Genetic work-up by WES identified a homozygous *PTCH1* nonsense mutation in the tumor tissue but not present in her blood cells or in non-lesional skin. In addition, heterozygous missense mutations were identified in three cancer-associated genes (*EPHB2*, *RET*, and *GALNT12*) in blood cells as well as in lesional and non-lesional skin. We also tested systemic immune therapy as a potentially beneficial approach to treat patients with numerous large BCCs on scattered areas of involvement. A rapid and sustained response to nivolumab was noted, suggesting that it is an efficacious drug for long-term therapeutic outcome.

Conclusion: *PTCH1*, *EPHB2*, *RET*, and *GALNT12* may potentially contribute to the synergistic oncogene driven malignant transformation manifesting as multiple, unusually large BCCs.

Keywords: immune therapy, malignant transformation gene-susceptibility, non-syndromic basal cell carcinoma, *PTCH1*, skin neoplasms

Introduction

Well-defined germ-line mutations in the *PTCH1* gene are associated with syndromic multiple basal cell carcinomas (BCCs) or Gorlin syndrome.¹ DNA isolated from the blood cells and non-sun exposed normal skin of our patient showed germ-line mutations of *GALNT12*, *RET*, and *EPHB2* (Tables 1 and 2). *GALNT12* is reported to be associated with increased susceptibility for colorectal carcinoma.²⁻³ *RET* is a known proto-oncogene reported in association with multiple endocrine neoplasia type 2, familial medullary thyroid carcinoma, lung carcinoma, pheochromocytoma, and other sporadic carcinomas.⁴⁻⁶ *RET* gene fusions caused by chromosomal rearrangements resulting in *RET* onco-protein have been associated with papillary thyroid carcinoma.⁷ Moreover, sequence

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Table 1**Documented cancer susceptibility and oncogenicity of mutations in *EPHB2*, *RET* and *GALNT12***

| Gene | Associated disorders |
|----------------|---|
| <i>EPHB2</i> | Prostate cancer, brain cancer |
| <i>RET</i> | Familial medullary thyroid carcinoma, Pheochromocytoma, Hereditary cancer-predisposing syndrome, Hirschsprung disease, Renal dysplasia, Multiple endocrine neoplasia types 1, 2a, 2b, and 4 |
| <i>GALNT12</i> | Hereditary cancer-predisposing syndrome |

Table 2**The GRCh38 coordinates of mutations in the present case with multiple large basal cell carcinomas**

| dbSNP ID | GRCh38 coordinates | | | Gene | Consequence | Amino Acid Change | 1000 genomes | | Mutant Allele Proportion Tissue Origin | | |
|-------------|--------------------|----------|---------|----------------|-------------------|----------------------------|--------------|----------|--|--------|--------|
| | Chr | Position | Ref Alt | | | | MAF | ExAC MAF | Blood | Normal | Lesion |
| rs28936395 | 1 | 22906853 | G A | <i>EPHB2</i> | nonsynonymous SNV | NM_004442:p.D679N (exon11) | 0.001198 | 0.0033 | 47% | 46% | 24% |
| rs77724903 | 10 | 43118460 | A T | <i>RET</i> | nonsynonymous SNV | NM_020630:p.Y791F (exon13) | 0.0002 | 0.0016 | 50% | 49% | 47% |
| rs145236923 | 9 | 98831947 | G A | <i>GALNT12</i> | nonsynonymous SNV | NM_024642:p.D303N (exon4) | 0.000799 | 0.0011 | 47% | 42% | 3% |

Chr, chromosome; Ref, reference sequence; Alt, alteration; MAF, minor allele frequency.

variants in *EPHB2*, another oncogene, were initially reported in prostate and brain carcinomas,⁸ but recently also in association with colorectal carcinoma⁹ and squamous cell carcinoma.¹⁰ Interestingly, high expression of oncogenes *GALNT12* and *EPHB2* is reported to be associated with favorable prognosis and survival in various malignancies, including follicular lymphoma and colorectal carcinoma. These studies have also suggested an association between lower expression of *GALNT12* and *EPHB2* oncogenes and poor prognosis in such patients.^{9,11-12} In the current study, we performed genetic tests on a female patient with numerous BCCs to identify the role of patched-1 in genetic mechanisms of this unusual presentation of the disease.

Material and methods

Patient

A 72-year-old woman with numerous primary BCCs involving non-sun exposed as well as sun-exposed areas of skin (Fig. 1) was enrolled in the study after the informed consent for performance of the genetic tests was provided.

Genetic tests

A peripheral blood sample, as well as skin biopsies from normal and tumor tissue were obtained from the patient, and DNA was extracted. These three DNA samples were sequenced on the Illumina NextSeq using the Illumina TruSeq Whole Exome Sequencing kit. Sequencing was performed in paired-end mode with 150-base reads. The resulting paired-end reads were aligned to the human genome version GRCh38, using the BWA mem aligner.

Alignment metrics indicated satisfactory coverage averaging above 100× for all three types of samples. Picard tools software was used to flag duplicates, and the genome analysis toolkit was used for base quality score recalibration; both steps are necessary to remove bias from subsequent variant calling. The FreeBayes Bayesian genetic variant detector was used to identify base substitutions and short insertion/deletion sequences in the samples. The resulting set of variants was annotated with ANNOVAR software. The presence and absence of variants and associated variant frequencies were reviewed across all three samples with aims of identifying (1) germline mutations in the genes with known roles in cancer etiology, and (2) somatic mutations or mosaicism in tumor sample with known roles in cancer development.

Results

Clinical report of the patient

The first BCC appeared when the patient was in her late 50s, and thereafter she developed about 10 new lesions per year. In her mid-60s, she noticed a sudden increase in appearance of new lesions as well as rapid increase in the size and volume of the existing tumors. Although at the beginning, few early lesions were treated surgically, later on she was no longer deemed to be a candidate for surgical interventions due to the size and number of tumors. When presented, she was suffering from pain and bleeding of numerous ulcerating lesions with major impact on her quality of life.

On physical examination, numerous skin tumors were observed mostly involving non-sun exposed areas (Fig. 1). Biopsies from several representative lesions confirmed the



Figure 1. Clinical presentation of the patient with numerous basal cell carcinomas before and after treatment with nivolumab. A–D: There are numerous large, exophytic, and ulcerating BCCs involving mainly non-sun exposed areas of the skin (A), including mid and lower back (B), bilateral thighs (C), and buttock (D), as well as sun-exposed areas including lower legs, forearms, and hands (not shown). Head and neck including face were largely spared. E–G: Almost all large and ulcerating tumors have regressed mostly or completely after treatment with nivolumab. There are few small and thin residual lesions that are gradually resolving after treatment.

diagnosis of BCC as the underlying malignancy (Fig. 2), while only one lesion showed squamous cell carcinoma. On careful examination, no other skin findings, such as palmar pits, suggestive of Gorlin syndrome, were noted.

Furthermore, whole body positron emission tomography/computed tomography scan failed to show any metastatic disease or extracutaneous findings suggestive of Gorlin syndrome (Fig. 2). Genetic profiling of DNA isolated from

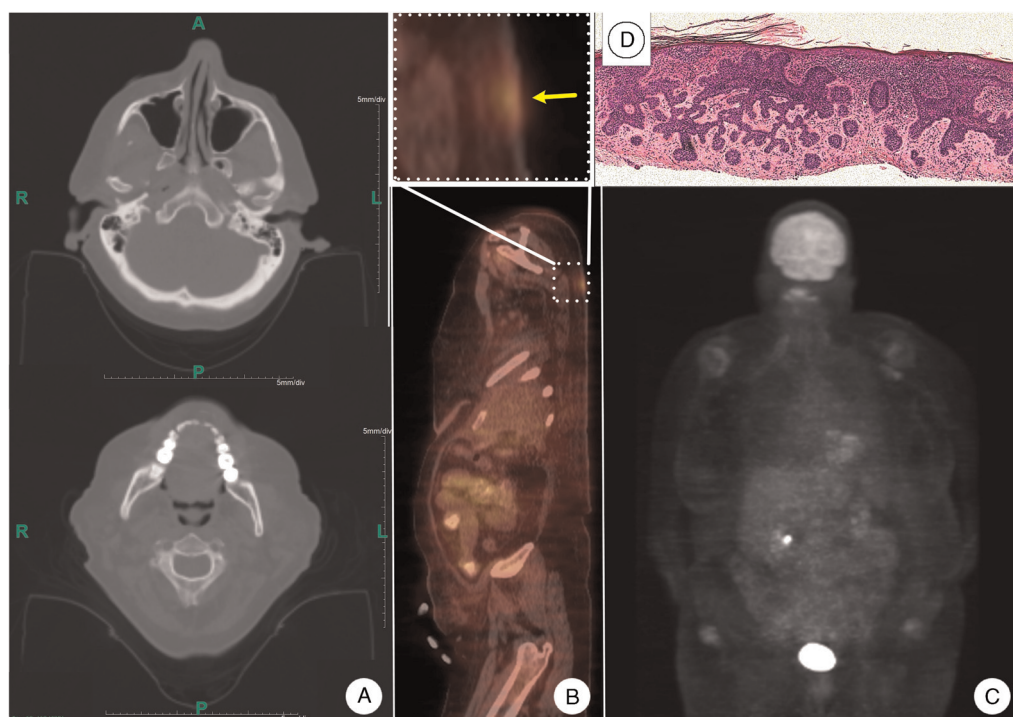


Figure 2. Imaging studies and histopathology. A: Head and neck CT scan failed to show any skeletal or brain stem abnormalities suggestive of Gorlin syndrome. B: Whole body PET/CT showed no evidence of metastasis (lower panel). Large cutaneous tumor (yellow arrow) showed increased metabolic activity (upper panel). C: Brain and other internal organs showed physiologic distribution of *fluorodeoxyglucose*. D: Histomicrograph of the biopsy from the lesion shown in section proved to be a BCC (hematoxylin-eosin stain). BCC: Basal cell carcinoma; CT: Computed tomography; PET: Positron emission tomography.

her blood cells and non-sun exposed normal as well as lesional skin identified mutations that can predispose to carcinomatous changes as detailed below in the Results and Discussion sections (Fig. 3). Given the extensive nature of her disease and possible underlying genetic predisposition, systemic treatment, including nivolumab, was considered.

PD-L1 and PD-1 protein expression has been reported in BCC and tumor infiltrating lymphocytes (TIL). In addition, anti-PD-1 inhibitors have shown to have activity in the treatment of advanced BCC.¹³ In a sample of the patient's BCC tumor, low positive PD-1 immunohistochemistry in TIL was reported by Foundation One Medicine. Therefore, nivolumab was started as an option given the complexity and the extent of her disease. Moreover, the long lasting effect of immune-modulating therapy approach was thought to help treat the existing lesions and hypothetically prevent recurrence once the treatment is discontinued.

Treatment was initiated with nivolumab 240 mg every 2 weeks. To enhance durable response, low-dose radiation was applied to one of the ulcerating tumors on her left back to elicit a neo-antigen response. The outcome proved successful with interim decrease in size and number of tumors on each subsequent visit (Fig. 1). However, after the ninth infusion, she developed grade 1 hepatitis and grade 2 colitis. Nivolumab was discontinued and the adverse events were successfully managed with a rapid course of tapering prednisone. Despite discontinuation of treatment, a year later the patient still exhibited a durable response showing interval improvement on each visit. All large ulcerating lesions had resolved and only a few small, thin BCCs remained (Fig. 1). Most importantly, no new lesions had been noticed since the first infusion of nivolumab.

Sequencing results

A total of 147,619 variants were annotated and subjected to bioinformatics filtering by steps shown in Fig. 3, and sequence variants in three documented cancer susceptibility genes were identified. These heterozygous germline variants, which were found both in the blood DNA as well as in non-lesional and lesional skin, included *EPHB2*: NM_004442: c.2035G>A, p.D679N; *RET*: NM_020630: c.2372A>T; p.Y791F; and *GALNT12*: NM_024642: c.907G>A, p.D303N. These sequence variants were confirmed by Sanger sequencing (Fig. 3). The proportions of mutant alleles of *EPHB2* and *RET* variants were high in all three types of DNA analyzed, while the *GALNT12* variant was present in lesional DNA at very low level (Tables 1 and 2). The p.D679N variant in *EPHB2* was previously associated with primary tumor of prostate cancer in 2 out of 95 primary and metastatic specimens.⁸ This variant resides in kinase domain of the *EPHB2* protein which is essential for receptor signaling and is the most frequently targeted region for mutations.

Next, the variant p.Y791F in *RET* has been reported in one patient out of 271 patients who presented with nonsyndromic pheochromocytoma without a family history of the disease.¹⁴ The third variant, p.D303N in *GALNT12*, has been previously reported in colorectal cancer and this mutation was shown to reduce the enzymatic activity of *N*-acetylgalactosaminyl transferase by 37%. In addition, the patient was found to have a homozygous, previously unreported sequence variant in exon 10 of *PTCH1*: NM_000264; c.1501C>T; p.Q501X. This mutation was present in tumor tissue only, and not in DNA isolated from blood cells or normal skin (Fig. 3). This sequence variant was absent in 119,654 alleles in the control population (ExAC.broadinstitute.org), and 1000 Genomes (www.1000genomes.org).

Discussion

In the case presented here, the presence of *GALNT12* and *EPHB2* was high in blood and normal non-sun exposed skin but drops to significantly lower level of presence in multiple samples of large, exophytic, and ulcerating BCCs from the same patient. Whether such findings reflect a parallel poor prognosis or the potential to advance the lesions to large exophytic and ulcerating tumors mainly in non-sun-exposed areas as seen in the present case, cannot be ascertained only on the basis of the one patient presented here.

It is plausible that patients who present with numerous skin carcinomas, but proven not to have Gorlin syndrome, may have a similar or another type of hereditary susceptibility to germ-line oncogene mutations that may involve areas as limited as a dermatome or the whole skin, depending on when such mutations occurred during embryonic development. This is in contrast with most patients in general population, who present with one or a few solitary basal cell carcinomas involving mainly sun-exposed areas of skin. Recently, such genetic susceptibility has been reported with the *ACTRT1* gene encoding an inhibitor of hedgehog signaling to suppress BCC. In our patient, no mutations in this gene were detected.

In published research from our group, the percentage of UV signature in idiopathic sun-induced SCC tumors ranged between 60% to 85%.¹⁵ In comparison, a BCC tumor sample from the shoulder of our patient, showed only 50% UV signature. There are published reports of >90% UV-specific p53 mutations in idiopathic cutaneous SCCs. In the same report, specific UV-induced mutations were found only in 50% of sporadic BCCs. These observations can potentially question the impact of cumulative UV irradiation as the main underlying driver etiology in formation of BCCs. When it comes to patients with numerous BCCs, especially in non-sun exposed areas, the UV causality in relation to BCC can be further questioned.¹⁶ In this context, new reports of BCC-associated genes, such as *PTPN14* and *LATS1* have recently been introduced in the literature.¹⁷

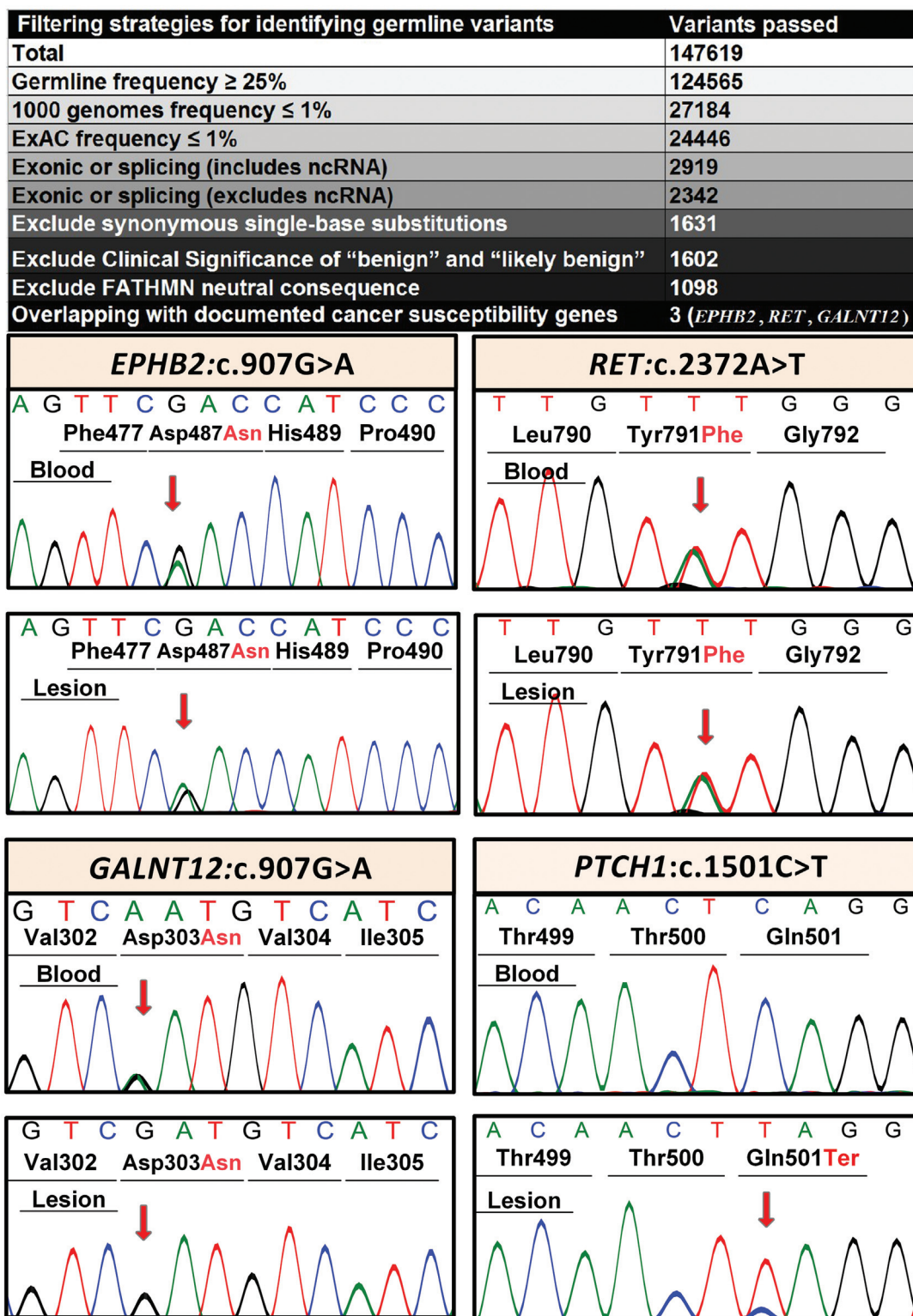


Figure 3. Identification of sequence variants in cancer susceptibility genes by whole exome sequencing. Filtering strategy for finding germ-line oncogene variants in DNA isolated from blood cells of the proband identified three candidate genes, including *GALNT12*, *RET*, and *EPHB2* (upper panel). Sanger sequencing confirmed the heterozygous state of these variants in blood and in lesional skin (lower panel). In addition, whole exome sequencing showed the presence of a homozygous *PTCH1* variant in the tumor sample while the blood cells and normal skin were negative for this variant.

One limitation is that the subject of the study was only one patient and we need to pursue these findings in additional cases with similar presentation. Also, we studied only two representative tumors among the numerous ones. Moreover, for control we used non-sun exposed normal skin, while in the future, we also plan to study adjacent normal skin as control.

Given the implication of such genetic predisposition, the patients could benefit from broader surveillance of skin as well as other organs at risk, including, but not limited to colon. Our patient had a recent normal colonoscopy as well as an updated normal mammography. Currently, she has full skin surveillance every 3–6 months. Extending genetic investigation to direct family members, especially offspring, can be helpful and potentially lifesaving. Accordingly, treatment options with durable response, such as immune modulating therapies, can be the preferred treatment method in skin carcinoma patients with underlying inherited genetic susceptibility for malignant transformation even in the absence of metastases. To the best of our knowledge, this is the first report of systemic immune therapy, nivolumab, given for treatment of BCC in general or specifically applied to a patient with BCCs with inherent susceptibility for malignancy transformation in the absence of metastasis.

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