Multiple cancer-related genes are mutated in p53 mutant patches in human skin

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INTRODUCTION

- Keratinocyte skin cancer (cutaneous squamous cell carcinoma (cSCC) and basal cell carcinoma) is the most common cancer globally.
- Sun-exposed skin contains numerous p53 immunopositive patches (PIPs, also known as p53 mutant patches) which are potential precursors of keratinocyte skin cancer.

It is known that PIPs contain *TP53* gene mutations, but unclear whether these lesions contain mutations of other cancer-related genes.

AIM

- Investigate for mutations in cancer-related genes within PIPs and compare with precancerous skin lesions (actinic keratoses (AK), Bowen's disease (BD)) and cSCC.

METHODS

- Freshly excised normal sun-exposed skin samples (containing PIPs on p53 immunostaining) and formalin fixed paraffin embedded (FFPE) precancerous skin lesions (AKs, BDs) and cSCCs.
- Following p53 immunostaining, PIPs and non-PIPs skin dissected under inverted light microscope.
- Laser capture microdissection used to isolate dysplastic and cancerous areas from FFPE tissue.
- Whole exome sequencing (WES) and targeted sequencing conducted with Illumina HiSeq 2000 platform and MiSeq system respectively.
- Data analysis performed using VarScan 2.3.3.

RESULTS

- WES of AKs showed median mutation burden of 34.5 (range 5.3–49.8) mutations per Mb. Analysis of mutated genes in WES of AKs and cSCCs, and present in COSMIC (Catalogue of Somatic Mutation in Cancer) database identified 18 genes for subsequent targeted sequencing in larger group of skin lesions (figure 1).
- Non-silent somatic mutations were identified in 13 of these 18 genes in PIPs (figure 2) and in 16 of 18 genes in AKs and/or BDs and most cSCCs adjacent to an AK or BD (figure 2).
- All 13 genes with non-silent mutations in PIPs were mutated in AKs, BDs and cSCCs in this study with some mutations identical between the different lesions (figure 3).
- PIPs contained clonally mutated and subclonally mutated genes, thus showing evidence of subclonal evolution in these lesions; in one case, 4 PIPs had originated from a shared precursor cell pool and evolved along a common mutation pathway before diverging into separate clones of cells (figure 4).
- Genetic evidence for development of cSCC from adjacent AKs and BDs was seen, thus providing genetic confirmation that AKs and BDs can develop into cSCC.

Figure 2: Targeted sequencing data of PIPs, AKs, BDs and cSCCs adjacent to AK or BD. (a) An example of a PIP within whole mount epidermis; table indicates size (area of epidermis) and number of p53 immunopositive cells within each PIP. (b-f) Heatmaps showing different mutation types within target genes in (b) PIPs, (c) AK, (d) cSCC adjacent to AK, (e) BD and (f) cSCC adjacent to BD. The corresponding bar graph on the right of each heatmap represents the mutation frequency of each of these genes. The frequency of silent and non-silent genetic alterations and type of base pair alteration within the samples is plotted above the heatmap. [adj = Adjacent].

Figure 4: Clonality and evolution of genetic alterations in PIPs, AK/cSCC and BD/cSCCs. (a) Four PIPs in p53 immunostained epidermis from individual skin sample. (b) Shared and new mutations in these 4 PIPs. (c) Absence of shared mutations in 2 PIPs in individual skin sample from another subject. (d-f) Shared and new mutations in adjacent lesions; (d) AK and cSCC, (e) BD and cSCC, (f) BD and cSCC. Evolutionary order of events inferred from variant allele frequency (VAF) of mutations in respective lesions.

CONCLUSIONS

- p53 immunopositive patches (PIPs) within chronically exposed human skin contain mutations in multiple cancer-related genes, with evidence of subclonal evolution in these lesions.
- Comparison of genetic mutations in cSCCs adjacent to AKs and in cSCCs adjacent to BDs provides genetic confirmation that AKs and BDs can develop into cSCC.

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