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509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Detection of Molecular Biomarkers in Fanconi Anemia Mucosa

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Introduction

Fanconi anemia (FA) is the most common cause of inherited bone marrow failure, and a cancer predisposition syndrome caused by impaired DNA interstrand crosslink repair, also known as the FA repair pathway. Hematopoietic stem cell transplantations (HSCT) have improved survival in patients with FA, however, those have also heightened the risk of malignancies. Head and neck squamous cell carcinoma (HNSCC), and specifically oral SCC (oSCC), are the most common solid malignancies and the leading cause of death in adult patients with FA. By 45 years of age, HNSCC has a computed cumulative incidence of 50% and 100% in FA patients who did not and did undergo a HSCT, respectively. Historically, SCC in FA has been mostly diagnosed at an advanced stage of disease conferring poor outcome. FA-associated HNSCC are known to be enriched with p53 and structural variants. We aim to develop molecular screening tools and biomarkers of oSCC development in FA patients, to facilitate measurement of potential prophylactic therapies and allow for earlier detection of premalignant oral lesions and cancer. To that end, we are mapping somatic genetic variants in normal-appearing oral mucosa as well as oral lesions in patients with FA, and we plan to evaluate circulating cancer biomarkers.

Methods

This is a proof-of-concept study conducted in healthy controls (HC) and FA patients. We use a non-invasive oral brush biopsy to collect oral keratinocytes from six different sites of normal-appearing mucosa: retromolar area, side of the tongue and floor of the mouth, bilaterally. DNA is isolated and the exonic regions of the *TP53* gene are sequenced using CleanPlex *TP53* Kit on an illumina Miseq platform, to identify single nucleotide variants (SNVs), insertion-deletions (indels) at an allele frequency above 1%. Genome-wide SNP genotyping with Infinium Global Diversity Array (v1.0) is used for copy number variant (CNV) identification. Areas of visible changes/abnormal appearing mucosa are also brushed and are tested for the aforementioned genetic changes, as well as for cytology and DNA ploidy. Circulating plasma and serum biomarkers will be evaluated at the end of the study.

Results

The study is ongoing. 20 HC and 17 FA patients have completed the study to date. Median age of the HC and FA cohorts were 36.3 years (range 23-61) and 31.8 years (range 15-50), respectively. Sex distribution was similar with males consisting of 44% and 53% in the HC and FA cohorts, respectively. The HC cohort included significantly more tobacco smokers and alcohol

consumers than the FA cohort (33.3% *vs.* 0 for smokers, and 83.3% *vs.*17.6% for alcohol consumers, in the HC *vs.* FA cohorts, respectively, $p<0.05$). Using an optimized DNA isolation protocol, we were able to obtain sufficient amount of high molecular weight DNA, for each of the six sites brushed. Both freshly collected and stored samples were successfully used for molecular studies. Storing of HC samples in SurePath preservation solution, for an average duration of 55 days, did not impair DNA yield or sequencing data. No somatic molecular changes were evident in the oral mucosa of the healthy controls (n=18) in over 100 samples taken. At least one somatic molecular change was evident in most participants with FA. Five out of the 15 participants (33.3%) in the FA cohort tested positive for 10 different clinically significant somatic *TP53* SNVs. At least one CNV was detected in samples sequenced from non-lesional oral mucosa in 10 out of 15 FA participants (66.7%), with numerous samples testing positive for multiple CNVs. The most frequently observed CNV was chromosome 9p isodisomy, observed in 6/15 participants (40%). Molecular analysis of 13 brushed lesions demonstrated 2 distinct clinically significant *TP53* somatic variants in one lesion and multiple CNVs in 3 lesions, all from one individual with FA. Cytology and DNA ploidy analyses were negative in 2 lesions, the third lesion showed suspicious cytology, resulting in a surgical biopsy, which was negative. Analysis of additional samples and recruitment of FA participants is ongoing.

Conclusions

This study validates noninvasive screening applications for identification of somatic molecular changes in the normal-appearing mucosa in patients with FA. Further studies will assess if these changes can be used as surrogate biomarkers of a short-term outcome for the evaluation of preventative treatment effectiveness in FA-associated oSCC and early diagnosis.

Disclosures No relevant conflicts of interest to declare.

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