Definition of Genome-wide cell-free DNA fragmentation in patients with oral cancer.

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**Abstract:**

**Background:** A recently defined novel approach by Christiano et al (2019) termed ‘DNA evaluation of fragments for early interception (DELPHI)’ demonstrated capacity to specifically differentiate and classify cancers originating in various organs by genome-wide analysis of cell-free circulating DNA (cfDNA) in plasma. Based on variability in length of the DNA fragments, unique patterns were characterized in association with various cancers originating in diverse tissues. Notably, higher quantities of smaller cfDNA have been associated with circulating cf tumor DNA. Further application of machine learning (ML) was shown to not only classify the pattern, but also project the tissue of tumor origin. Detection rate of patients with cancer was over 90%. The current study proposes to extend application of DELPHI to oral cancer, which has not been previously investigated. Meanwhile, we extent traditional DELPHI to DNA methylation based DELPHI (meDELPHI) with non-bisulfite methylation sequencing technique. When identified in early stages, oral cancer is highly treatable with good survival outcomes. However, due to lack of reliable biomarkers that detect malignant transformation, oral cancer is often diagnosed only at late stage disease, which is associated with poor prognosis, high morbidity and mortality. The study proposes to examine whether DELPHI analysis of plasma has applicability for distinguishing individuals between oral cancer, healthy oral tissue and leukoplakia with high fidelity.

**Hypothesis:** Investigators propose that meDELPHI analysis may have potential utility for screening, monitoring and early detection and of malignant transformation in patients with oral leukoplakia.

**Approach:** A prospective pilot study design proposes enrollment and collection of blood from: 1) healthy subjects, 2) individuals with leukoplakia and 3) individuals with oral cancer at time of diagnosis. Following extraction of cfDNA from plasma, sequencing libraries will be created and subjected to whole genome sequencing. The ratio of small (100-150 base pairs (bp) to large (151-200 bp) cfDNA fragments distribution and specific position in the genome will be mapped in order to create distinctive profiles found in healthy patients, those with leukoplakia and those with oral cancer. Genome mapping will proceed by characterizing cfDNA fragments in 5 mega-base segments across the length of the genome, generating approximately 20,000 reads/segment at 1-2X genome coverage. ML will be applied to: 1) classify individuals based on their oral health status and 2) confirm oral cavity tissue as the tissue of origin in subjects with oral cancer.

**Significance/Impact**: This study will determine whether DELPHI is a feasible approach for distinguishing oral cancer from oral leukoplakia and healthy oral tissue. Further, this study will identify genomic loci associated with oral cancers based on definition of genomic segments exhibiting higher ratios of small cfDNA. This study has high translational potential for screening patients at risk for oral cancer and early detection of malignant transformation if DELPHI exhibits high sensitivity and specificity in the context of oral cancer. Pending outcome, this study will collect preliminary data for a larger prospective study to validate efficacy of this approach as a potential screening tool for monitoring and detecting early-stage oral cancer in the clinical setting and process pilot for future clinical trial development.

Reference:

Bisulfite-free methylation sequencing: <https://www.nature.com/articles/s41587-019-0041-2>