Ultra-Deep Sequencing of Cell-Free DNA for Screening and Monitoring Gynecological Cancers

Cassie Schumacher1, Naya Nohl2, Olga Camacho-Vanegas3, Jonathan Irish1, Suhindar Sandhu1, Laurie Kurihara1, Peter Dottino1, Melissa Schwartz2, Timothy Harkins1, John Martingilli2, Vladimir Makarov1

1Swift Biosciences, 58 Parkland Plaza, Suite 100, Ann Arbor, MI 48103
2Icahn School of Medicine at Mount Sinai, Departments of Genetics and Genomic Sciences and Obstetrics/Gynecology & Reproductive Sciences, 1425 Madison Avenue, New York, NY 10029

Abstract

Axcellar DNA in blood and other bodily fluids is known cell-free DNA (cfDNA). cfDNA arises from apoptosis or necrosis of healthy and cancerous cells. For cancer, noninvasive collection and analysis of cfDNA can be used to screen for tumor-derived mutations. Here we present a method using targeted, PCR-based next generation sequencing (NGS) to identify low frequency somatic mutations in cfDNA from women with uterine and ovarian cancers. In two pilot studies we demonstrate how ultra-deep targeted sequencing enables variant detection down to 1%. This technique also includes amplicons which target germline SNPs to ensure proper sample tracking by generating each individual’s unique genetic fingerprint. The first study was a retrospective examination of circulating cfDNA from blood while the second study examined cfDNA derived from uterine lavage as a potential screening method to detect early stage uterine cancer.

In the first study, 11 women had previously undergone tumor resection and the tumor profile was determined using an Accel-Accord panel targeted to 36 oncology-related genes. cfDNA samples were then isolated from blood at two or more time points ranging from 7 to 46 months apart, and sequenced using the same NGS-baselined amplicon panel. In the second study, 104 women underwent uterine lavage. cfDNA and DNA derived from cellular material were isolated from the sequenced lavage. The samples were then sequenced with an amplicon-based narrow-amplicon panel that represented a subset of the previous panel, focusing only on the gynecological-related oncology genes. In the first study, tumor-specific mutations were found in the cfDNA of 8 of 11 women. In one case, the frequency of mutation detected in the cfDNA was nearly three times that detected in the tumor, and the patient died 4 days later. In the second study, 7 women were identified by histopathology to have uterine cancer, and in paracrine oncology-related mutations were discovered in all of the corresponding cfDNA samples. Additionally, 51 of the 104 women not identified by histopathology to have cancer had cfDNA bearing oncology-related mutations. All mutations in this study were further validated using digital droplet PCR.

We have provided preliminary evidence for using ultra-deep sequencing of cfDNA derived from both blood and uterine lavage fluid as a means of screening for and monitoring gynecological cancers in the research setting.

Uterine Lavage and Accel-Amplicon Sequencing

Figure 4. A) Depiction of the study pipeline. Following uterine lavage, samples were processed and cell pellet and cfDNA isolated. For more details, please see Nair et al., PLoS Med 2010. Accel-Amplicon libraries were then made using a panel comprised of the genes in B) Mutations detected by NGS were then validated with either Sanger Sequencing or ddPCR and then the mutations were class analyzed. C) Patient enrolment and number of samples in each study.

Mutation Distribution Detected by Accel-Amplicon

Figure 5. Patients are shown along with the genitourinary studies (top bar), mutations detected for each gene and by total mutation number (second line). Mutations detected in uterine lavage samples are color-coded hierarchically (driver, potential driver, passenger). If ≥ 1 mutation was detected per gene and in the same patient, the most consequential one is shown. NGS-detected mutations validated by ddPCR or Sanger are represented by black dots.

Cancer Type: Uterine Cancer

Figure 6. Total number of mutations detected in gene hotspots in this study and in endometrial tumors studied by TCGA. The percentage of samples affected are given in parentheses. *Mutations in hotspot positions detected, respectively, in lavage and TCGA tumors are given in parentheses; novel mutations detected by sequencing are highlighted in bold.

Mutation Classification

Figure 7. Patients are listed from left to right in decreasing order of the sum of driver and potential driver mutations.

Conclusions

| 10 ng of tumor DNA, normal DNA isolated from blood, and cell pellet and cfDNA isolated from uterine lavage fluid can be used to identify somatic variants present in the peripheral blood and uterine lining.
| Sample ID allows samples derived from the same individual to be properly paired during analysis.
| Sequencing cfDNA derived from women with gynecological cancer can identify the same variants seen in the tumors and at various times after resection.
| There is an observed correlation between recurrence of tumor mutations in the cfDNA of individuals with gynecological cancers and outcome. More studies would be done with frequent and consistent point times to further probe the utility of this technique.
| Accel-Amplicon can be used to prospectively detect early-stage, microscopic endometrial cancer.
| NGS-based analysis of uterine lavage can achieve the necessary sensitivity for endometrial cancer screening.
| More experiments are needed to understand the impact of detected mutations on endometrial cancer development.
| Massively parallel sequencing using Accel-Amplicon enables detection of novel mutations.

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58 Parkland Plaza, Suite 100 - Ann Arbor, MI 48103
www.swiftbiosci.com

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