Can Mitochondrial DNA Mutations Be Used as a Biomarker for Endometrial Cancer?

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Introduction

Endometrial cancer, also known as EC, had 417,000 new cases in 2020 and is the 6th most common cancer in women and the 15th most common cancer overall (wcrf.org). Endometrial cancer affects the endometrium, the inner lining of the uterus. When endometrial cancer occurs, something causes changes, or mutations, inside the endometrium's cell's DNA. This causes healthy cells, which grow, multiply, and die at a fixed rate, to mutate into abnormal cells, which do not die at a set time and will grow and multiply uncontrollably. The buildup of these abnormal cells creates a mass (tumor) (mayoclinic.org). In addition, damaged or defective DNA can change important genes that control cell growth. When the genes are damaged, this can cause uncontrollable growth that could cause cancer (cancer.org). Our project looked at mitochondrial DNA (mtDNA) templates derived from blood serum DNA extracts from EC patients to determine if mutations can be detected in the blood.

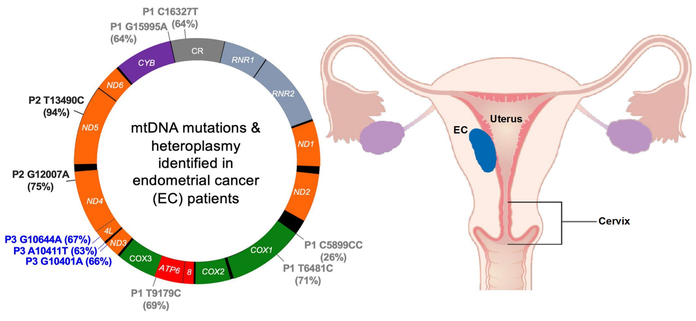

Figure 1. mtDNA mutations & heteroplasmy identified in three EC patients. Variants for patients 1, 2, and 3 are indicated on the outside of the map by P1, P2, and P3, respectively, and the percentage heteroplasmy for each mutation is reported.

Modified from, https://en.wikipedia.org/wiki/Endometrial_cancer and https://doi.org/10.3390/life12040562.

Methods

Starting PCR

TC Water, MM, Fwd/Rev Primers, and template were added.

Running PCR

Number of cycles varied on the amount of template used.

Electrophoresis

1% agarose (6.1 V/cm) for 1.5hrs & 1.2% agarose (6.8 V/cm) for 1.6hrs were used.

Extraction

Amplicon extraction was done using the E.Z.N.A. kit.

Quantification

DNA was quantified using Qubit 1X dsDNA HS Assay Kit.

TC water - Tissue Culture Water
mtDNA - mitochondrial DNA

PCR - Polymerase Chain Reaction
MM - Master Mix

Results

Table 1 . EC tumor-specific DNA templates, and primers used to add Next-Generation Sequencing barcodes to PCR amplicons.

Patient & Mutation	Primers used for PCR	Template DNA (ng)	PCR Cycle No.[a]	Amplicon Conc. (ng/µl)	Total DNA (ng)[b]
G10401A, Patient 3	For 7, Rev 7	10	15	8.53	298.6
T146C, Patient 3	For 6, Rev 6	10	15	32.53	1138.6
C16270T, Patient 2	For 8, Rev 8	10	15	8.58	300
G10644A, Patient 3	For 12, Rev 12	10	15	24.01	840.4
G12007A, Patient 2	For 9, Rev 9	21	8	12.72	445.2
G15995A, Patient 1	For 3, Rev 3	10	15	22.43	785.1
T9179C, Patient 1	For 2, Rev 2	10	15	33.09	1,158.20
C16327T, Patient 1	For 11, Rev 11	10	15	17.04	596.4
T16298C, Patient 1	For 5, Rev 5	12	15	17.84	624.4
T6481C, Patient 1	For 1, Rev 1	21	10	16.27	569.5

a All PCRs were done using Phusion DNA polymerase with GC buffer (F-532L). PCR Conditions: 98 degrees for 30 seconds, (98 degrees for 10 seconds, 62 degrees for 30 seconds, 72 degrees for 30 seconds)x8-15 cycles, 72 degrees for 5 minutes, 4 degrees for holding.
b Following gel extraction, all samples were eluted with 35 µl of TC water.

Table 2. Primers for Amplicon Next Generation Sequencing.

Pair	
Forward 1	AATGATACGGCGACCACCGAGATCTACAC AACCCCTC ACACTCTTTCCCTACACGAC
Forward 2	AATGATACGGCGACCACCGAGATCTACAC TCCGCGAA ACACTCTTTCCCTACACGAC
Forward 3	AATGATACGGCGACCACCGAGATCTACAC AACCCCTC ACACTCTTTCCCTACACGAC
Forward 5	AATGATACGGCGACCACCGAGATCTACAC CAGATCCA ACACTCTTTCCCTACACGAC
Forward 6	AATGATACGGCGACCACCGAGATCTACAC CGTACTAG ACACTCTTTCCCTACACGAC
Forward 7	AATGATACGGCGACCACCGAGATCTACAC CTCTCGTC ACACTCTTTCCCTACACGAC
Forward 8	AATGATACGGCGACCACCGAGATCTACAC GTAGAGGA ACACTCTTTCCCTACACGAC
Forward 9	AATGATACGGCGACCACCGAGATCTACAC CAGATCCA ACACTCTTTCCCTACACGAC
Forward 11	AATGATACGGCGACCACCGAGATCTACAC AAGAGGCA ACACTCTTTCCCTACACGAC
Forward 12	AATGATACGGCGACCACCGAGATCTACAC ACTTGACG ACACTCTTTCCCTACACGAC
Reverse 1	CAAGCAGAAGACGGCATACGAGAT GTCCGAGG GTGACTGGAGTTCAGACGTGT
Reverse 2	CAAGCAGAAGACGGCATACGAGAT ATAGAGAG GTGACTGGAGTTCAGACGTGT
Reverse 3	CAAGCAGAAGACGGCATACGAGAT CATCCGAA GTGACTGGAGTTCAGACGTGT
Reverse 5	CAAGCAGAAGACGGCATACGAGAT CGGAGAGA GTGACTGGAGTTCAGACGTGT
Reverse 6	CAAGCAGAAGACGGCATACGAGAT CGCTATGT GTGACTGGAGTTCAGACGTGT
Reverse 7	CAAGCAGAAGACGGCATACGAGAT TCTGTTGG GTGACTGGAGTTCAGACGTGT
Reverse 8	CAAGCAGAAGACGGCATACGAGAT TAGCCGCG GTGACTGGAGTTCAGACGTGT
Reverse 9	CAAGCAGAAGACGGCATACGAGAT TAGCCGCG GTGACTGGAGTTCAGACGTGT
Reverse 11	CAAGCAGAAGACGGCATACGAGAT AGCTAGAA GTGACTGGAGTTCAGACGTGT
Reverse 12	CAAGCAGAAGACGGCATACGAGAT CTAGTCGA GTGACTGGAGTTCAGACGTGT

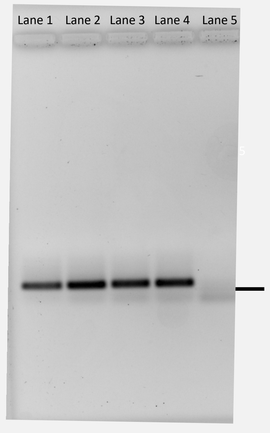

Figure 2. G10401A gel. Lanes 1-4 G10401A amplicon scaled up for extraction, & lane 5 had negative control [contained no template].

Next Generation Sequencing

After quantification, the DNA was sent off to our collaborator for **Next-Generation Amplicon Sequencing**, which will detect mutations of the mtDNA. We are still currently awaiting the results.

Discussion

Next-generation amplicon sequencing revealed that at least one tumor-specific heteroplasmic mtDNA mutation is detectable at ≥0.35% using our method. The results from this research could present a non-invasive testing option for those with endometrial carcinoma: a simple blood test to search for mtDNA mutations. This would not only be non-invasive but also make it easier to test for EC regularly. This could mean possibly catching cancer that has recurred earlier and offering a higher chance of remission, as EC is most likely to recur in the first few years after treatment (Young *et al*.). In addition, certain types of EC and cancer treatments have been connected to being at an elevated risk of select second cancers, such as breast, colon, rectal, lung, lymphoma, bladder, kidney, vaginal, soft tissue, and acute leukemia (cancer.org). Looking at the big picture and future research, if the results were to confirm that mtDNA mutation can be used as a biomarker, there could be fine-tuning of the process we used. There could also be research looking at expanding into being able to detect other types of cancers with this or a similar technique.

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