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Short communication

Single nucleotide variations in cultured cancer cells: Effect of mismatch repair

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ABSTRACT

We assessed single nucleotide variations (SNVs) between individual cells in two cancer cell lines; DU145, from brain metastasis of prostate tumor with deficient mismatch repair; and HT1080, a fibrosarcoma cell line. Clones of individual cells were isolated, and sequenced using Ion Ampliseq comprehensive cancer panel that covered the exomes of 409 oncogenes and tumor suppressor genes. Five clones of DU145 and four clones of HT1080 cells were analyzed. We found from 7 to 12 unique SNVs between DU145 clones, while HT1080 clones showed no more than one unique SNV. We then sub-cloned individual cells from some of these isolated clones of DU145 and HT1080 cells. The sub-clones were expanded from a single cell to approximately one million cells after about 20 cell divisions. The sub-clones of DU145 cells had from one to four new unique SNVs within the sequenced regions. No unique SNVs were found between sub-clones of HT1080 cells. Our data demonstrate that the extent of genetic variation at the single nucleotide level in cultured cancer cells is significantly affected by the status of the DNA mismatch repair system.

1. Introduction

Cultured cancer cell lines provide valuable information about molecular mechanisms of cancer that is often hard to obtain by *in vivo* studies. Studies of cultured cancer cells helped to establish chromosomal abnormalities, high mutation rates, and genetic instability in the cancer cell populations (reviewed in [1]). With advent of the next generation sequencing (NGS), it became possible to extend studies on genetic variability in cancer cells to the level of single nucleotide variations (SNVs) [2–5]. Thus, it has been established that *in vivo* cancer tumors consist of multiple genetically distinct cell populations; however, such information for the cultured cancer cells is incomplete.

In this pilot study, we assessed single nucleotide variations in two cancer cell lines, DU145 and HT1080. The prostate cancer-derived DU145 cell line with deficient mismatch repair (MMR) exhibited the highest mutation rate among all of the MMR-deficient cell lines [6]. In contrast, fibrosarcoma HT1080 is a near diploid cell line with wild type p53, and a relatively stable genome among the cancer cell lines [7].

DU145 cells showed heterogeneity in expression of cancer [8] and epigenetic [9] markers. They also exhibited chromosomal instability and karyotype heterogeneity, which were demonstrated by spectral karyotype analysis [10]. It was found that defects in MMR resulted in a significant increase in the rate of spontaneous mutations [6]. Recently, it was shown that MMR is responsible for variation in the somatic mutation rate across the human genome [11].

Herein, we extended genetic variability studies in cancer cell lines to SNVs analysis using NGS. To assess SNV between individual cells in the original cultures we isolated five clones of DU145 and four clones of HT1080 cells and analyzed them with targeted NGS. The targeted comprehensive cancer panel (CCP) covered exomes of 409 oncogenes and tumor suppressor genes. Even within this limited portion of the genome, we found a significant number of SNVs between individual clones in DU145 cells. The sub-clones derived from these clones also have detectable number of SNVs. In contrast, HT1080 cells showed an insignificant number of SNVs within the target regions.

2. Materials and methods

DU-145 and HT-1080 cell lines were obtained from ATCC and were grown to 50% confluency according to ATCC recommendations. Cells were collected by Trypsin/EDTA and seeded in 10 cm cell culture dishes at approximately 50 cells per dish. After 14 days, visible distinct colonies generated from single cells were collected using sterile cloning cylinders (Bel-Art–SP Scienceware, South Wayne, NJ) and the manufacturer's protocol. Collected colonies were transferred to separate plates for further growth. When the total number of cells in cultures reached approximately 1×10^6, they were collected and DNA was purified for sequencing.

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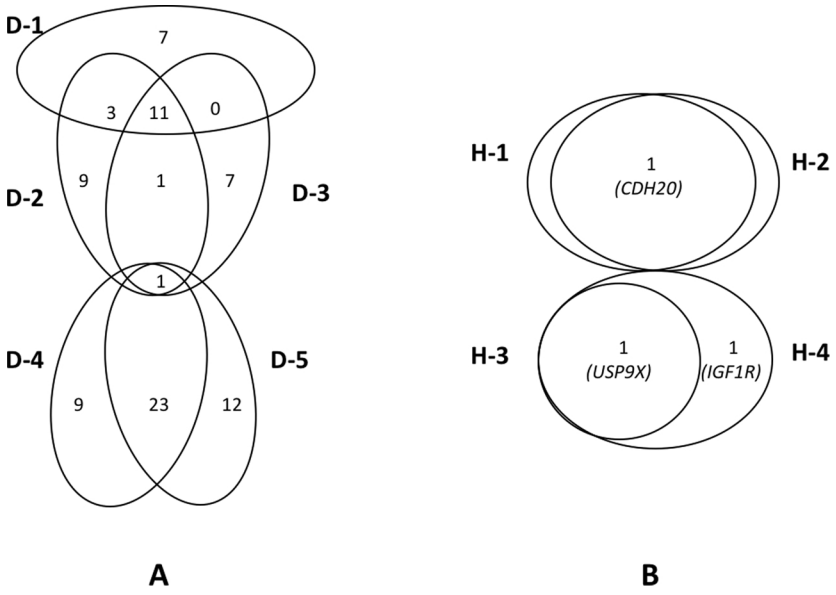

Fig. 1. Venn diagram shows the numbers of SNVs in DU145 (A) and HT1080 (B) clones that were unique for a particular clone or shared by several but not all the clones.

Genomic DNA was isolated using a PureLink® Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) and quantified by a NanoVue Plus spectrophotometer (GE Healthcare Life Sciences, Piscataway, NJ, USA). A total of 50 ng of DNA of each sample was subjected to PCR amplification of target regions using 16000 primer pairs in four pools according to Ion AmpliSeq™ Comprehensive Cancer Panel that covers exons of 409 genes (Thermo Fisher Scientific). Barcode adaptors from the Ion Xpress™ Barcode Adapters Kit (Life Technologies, Grand Island, NY, USA) were ligated to the amplicons to allow for sample multiplexing. Sequencing libraries were prepared using the Ion AmpliSeq™ Library Kit 2.0 (Thermo Fisher Scientific) as instructed by the manufacturer. All prepared libraries were quantified using RT PCR based Ion Library TaqMan Quantitation kit (Thermo Fisher Scientific).

Each uniquely barcoded library was diluted in nuclease-free water to a stock concentration of 100 pM. The samples were prepared for sequencing using Ion Chef system and Ion PGM Hi-Q Chef Kit, and were loaded onto Ion 318™ chips (Thermo Fisher Scientific). Semiconductor-based NGS was performed on an Ion PGM™ system (Thermo Fisher Scientific) using Ion PGM™ Hi-Q™ Sequencing Kit. The successful sequencing of a sample set was determined based on the manufacturer's recommendations of expected throughput and total number of reads with a quality score of AQ20 (one misaligned base per 100 bases) for each chip.

Base calling and alignment of sample sequence to the reference genome (hg19) were performed by the Torrent Suite™ software version 5.0.2 (Thermo Fisher Scientific). All sequencing reads were automatically barcode-sorted with low-quality reads removed. The resulting BAM files were uploaded to Ion Reporter software on Thermo Fisher Cloud server for variant analysis. Variants were annotated using AmpliSeq CCP single sample-PGM 5.0 Ion Reporter application. Variant differences between clones were identified by using a custom workflow based on Ion Reported AmpliSeq CCP tumor-normal application. The variants were further filtered using the following filters: homopolymer length ≤ 7, variant type = SNV, allele read-count ≥ 20, alternate allele count ≥ 20. SNV frequency was measured as a ratio of the reads with the alternative allele to the total number of reads covering the genomic position of the SNV.

In parallel, NextGENe software package version 2.3.4 (SoftGenetics, State College, PA, USA) was also used for analysis of SNV differences between clones. Clones were compared pairwise as tumor-normal pairs using somatic mutation comparison option with the following parameters: maximum contamination = 0.5%; somatic allele count = 20; relative directional balance (T/N) = 3.0; somatic allele frequency ration (T/N) = 1.5.

Additional analysis included visual inspection of the read alignment and the presence of nucleotide variants on the Integrative Genomics Viewer (IGV, Broad Institute, Boston, MA) to confirm the variant calls by checking for possible sequencing errors. Gene Ontology analysis was done using online PANTHER Classification System (release 11.1; panterdb.org).

3. Results

The original cultures of DU145 and HT1080 cells were grown to 50% confluency. The cells were collected by Trypsin/EDTA and seeded into 10 cm cell culture dishes at approximately 50 cells per dish. Colonies of individual clones were isolated as described in the methods section. We selected five DU145 clones (D-1–D-5) and four HT1080 clones (H-1–H-4) for targeted sequencing. When compared with the reference human genome (hg19), DU145 clones contained 1120 common SNVs within the targeted region with frequencies greater than 10%, including 126 SNVs listed in the Catalogue of Somatic Mutations in Cancer (COSMIC) database. Interestingly, our targeted sequencing detected mutations in both *MLH1* and *PMS2* genes that were identified in Ref. [6] as the reason for the dysfunctional MMR in this cell line. The corresponding numbers of SNVs for HT1080 cells were slightly lower; their clones contained 945 common SNVs with frequencies more than 10%; of that, 93 SNVs were listed in the COSMIC database.

To identify SNVs that were not common in all the clones, their sequences were compared pairwise using tumor-normal algorithms of both Ion Reporter and NextGene software packages to identify SNVs. Based on these analyses for DU145 cells, a table was compiled that showed SNVs that were not present to all the clones but either unique for a particular clone or shared by several but not all the clones. (See Supplementary Table S1 in the online version at DOI: http://dx.doi.org/10.1016/j.mrfmmm.2017.07.003).

The results are also presented as Venn diagrams in Fig. 1. Five clones of DU-145 cells fall into two groups. One group consists of clones D-1, D-2, and D-3 that have 11 shared SNVs and 7, 9, and 7 unique SNVs, correspondingly. Another group consists of clones D-4 and D-5 with 23 shared SNVs, and 9 and 12 unique SNVs, respectively. There was only one shared SNV between these two groups that was common in clones D-2, D-3, D-4 and D-5. The clones of HT-1080 cell line had considerably less SNVs between them than the clones of DU-145 cell line (Fig. 1). HT-1080 clones had at most one SNV; only clone H-4 had a