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Cancer cells possess different isotopic enrichment: Isotopic induced functionalizations of normal DNA mutations leading to cancer

Reginald B. Little [a,*], Maiya Snowton [a], Orit Uziel [b]

[a] Department of Natural Science, Stillman College, Tuscaloosa, AL, USA
[b] The Felsenstein Medical Research Center, Rabin Medical Center and Faculty of Medicine, Tel-Aviv University, Israel

A B S T R A C T

Although the dynamics of telomeres during the life expectancy of normal cells has been extensively studied, there are still some unresolved issues regarding this research field. For example, the conditions required for telomere shortening leading to malignant transformations are not fully understood. In this work, we mass analyzed DNA of normal and cancer cells for comparing telomere isotopic compositions of white blood cells and cancer cells. We have found that the 1327 Da and 1672 Da characteristic telomere mass to charges cause differential mass distributions of about 1 Da among normal cells relative to cancer cells. These isotopic differences are consistent with a prior theory according to which replacing primordial, common isotopes of ^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{24}Mg, ^{31}P and/or ^{32}S by nonprimordial, uncommon isotopes of ^{2}D, ^{13}C, ^{15}N, ^{17}O, ^{25}Mg and/or ^{33}S leads to altered enzymatic dynamics. This replacement may subsequently modulate DNA and telomere codons resulting in transformation of normal cells to cancer cells (in 15 N depletion in telomeres dependent manner). The prior theory and the current data are consistent also with a recently observed non-uniform methylation pattern of the DNA of cancer cells relative to a more uniform methylation in the DNA of normal cells. We observe further evidence of nonprimordial isotopic accelerations of acetylations, methylations, hydroxylations and aminations of nucleosides with alterations of phosphorylations of nucleotides; which may explain the induction of mutations at the DNA, RNA and proteins leading to cancer and more general alterations of DNA, which are associated with aging. This difference in mass spectra between normal and cancer DNA may stem from different functionalizations and isotopic enrichments affecting the motion derived from nuclear magnetic moments (NMMs). We suggest that this phenomenon may lead to malignant transformation.

1. Introduction

The functions of the DNA replications, RNA transcriptions and protein translations are altered intrinsically due to mutations, including the abnormal replacements of nucleotides. In normal cells, mutations are mended by DNA repair mechanisms and due to the protective function of telomeres with proper lengths. When telomeres are too short, they can no longer fulfill their protective functions and therefore mutations accumulate [1]. If some of these mutations are cancerous and telomerase (the enzyme that prevents telomere shortening) is upregulated, a tumor might develop [2]. Currently, it is not fully known what leads to the accumulations of mutations in the DNA and the assumption is that these are random processes. Recent observations correlated mutations in driver genes, either upregulation of oncogenes or the downregulation of tumor suppressors, to the development of cancer [3].

Here we suggest that isotopic induced functionalizations with clumping of isotopes of nonzero nuclear magnetic moments (NMMs) or non-primordial ones induce the formation of cancer related mutations. For example, replacement of common, primordial: ^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{24}Mg, ^{31}P, ^{32}S, and ^{40}Ca with corresponding uncommon, nonprimordial: ^{2}D, ^{13}C, ^{15}N, ^{17}O, ^{25}Mg, ^{33}S, and ^{43}Ca may cause

* Corresponding author. Stillman College, 3601 Stillman Blvd., Tuscaloosa, AL, USA.
E-mail addresses: rlittle@stillman.edu, redge_little@yahoo.com (R.B. Little).

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formations of mutations in the DNA level either directly or via altered DNA repair mechanisms. Such replacements may be quantified by the ratios: $^{2}D/^{1}H$; $^{13}C/^{12}C$; $^{15}N/^{14}N$; & $^{17}O/^{16}O$. In this study, the objective is to measure isotopic ratios by mass spectrometry for possibly understanding isotopic effects for causing telomere malfunctions from normal shortening to lengthening for normal cells to transform to cancer cells.

A previously published theory has proposed another explanation for the formations of mutations. According to this theory, isotopes in enzymes can manifest different chemical and enzymatic properties due to magnetism at nano, molecular and atomic sizes [4,5]. Since isotopes have the same number of protons but different numbers of neutrons, it has been thought that isotopes have the same chemical properties for a given element. But based on the prior theory [4,5], enzymes were proposed to distinguish between isotopes by NMMs; as clumped non-zero NMMs were proposed to transiently alter enzymatic structures, properties and dynamics affecting their activities.

Our study provides further consistency of the previous theory [4,5] and the observed selective sensitivity of cancer cells to the nonprimordial isotopes (of ^{25}Mg, ^{43}Ca and ^{67}Zn) versus normal cells sensitivity. This may explain also the lack of sensitivity to the nonprimordial isotopes of ^{24}Mg, ^{40}Ca, and ^{64}Zn of cancer and normal cells [6].

2. Results

The results of the mass spectroscopy analysis are shown in the following Figs. 1 and 2 and in Supplementary Figures I, II and III. Figs. 1 and 2 are the original data. Supplementary Figures II and III specify mass intensities of importance of Figs. 1 and 2. This data reveals the general manifestations and reproducibility of the isotopic enrichment phenomena in many different cancers with emphasis on differing isotopic enrichments of the cancer telomeres relative to telomeres from normal cells. The observed masses correspond to fragmentations of the DNA from the cancer cells, normal B-lymphocytes and total white blood cells. The fragmentations are induced by the energy of laser in the mass spectrometer ablating the DNA; such laser fragmentations are analogous to enzymatic and gravitational induced alterations of the DNA. Ease laser fragmentation is related to the activity of enzymatic fragmentations. By analyzing the DNA

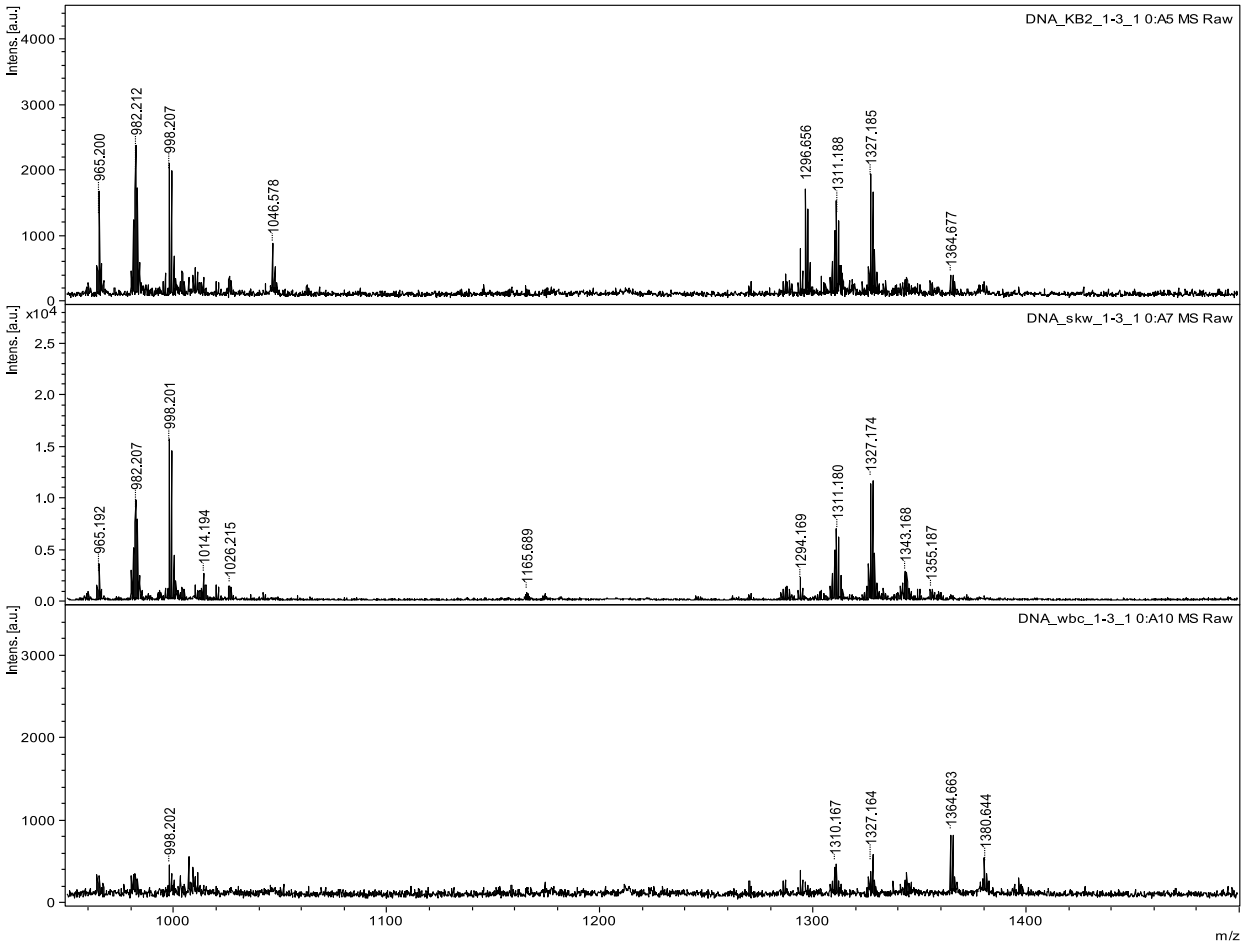

Fig. 1. Mass Spectra from 975 Da to 1500 Da with telomere $\{G_3(T_2AG_3)_3\}^{5+}$ at 1327 Da: A spectrum of the cancer cells is denoted by K562; Lymphocytes cells are marked as DNA SKW; White Blood Cells are depicted as DNA WBC.

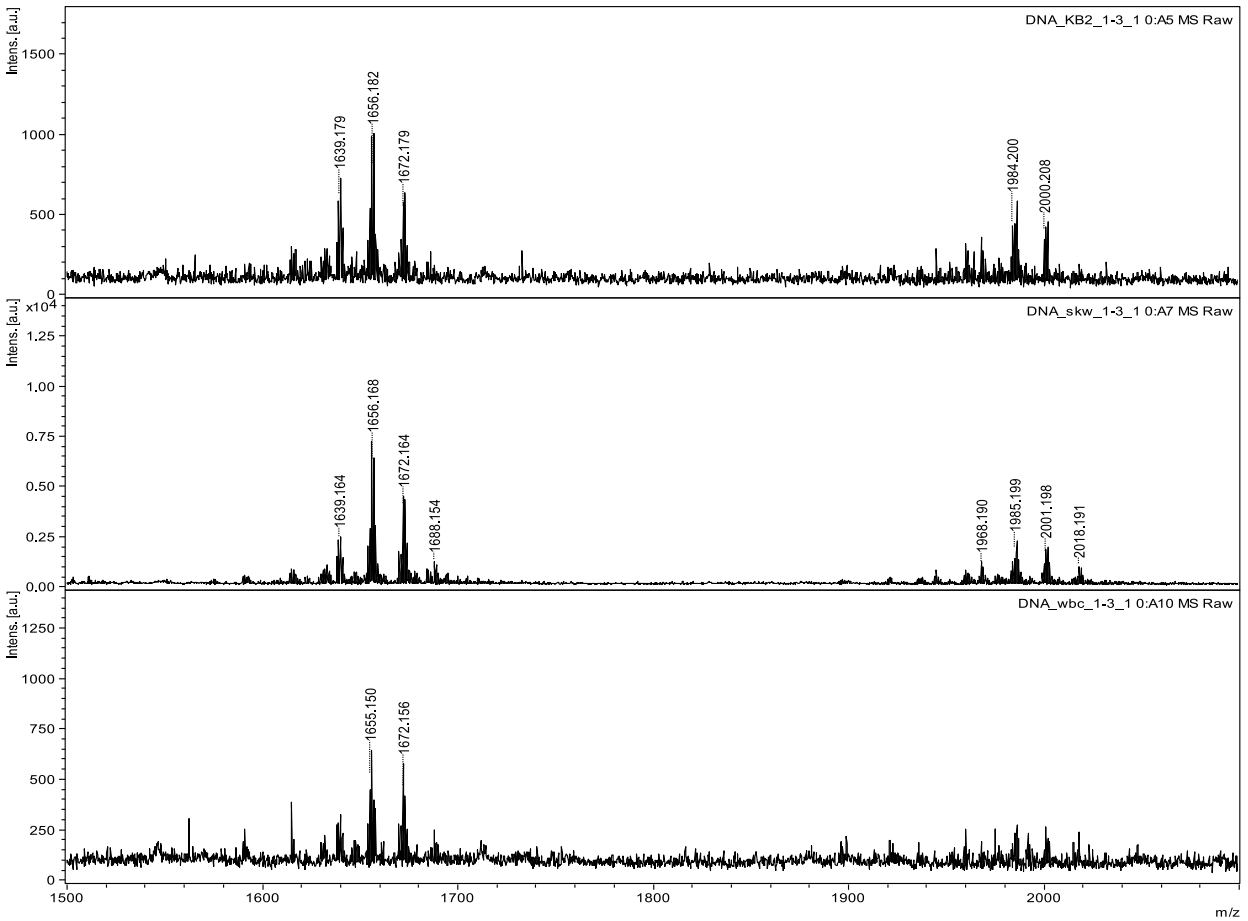

Fig. 2. Mass Spectra from 1500 Da to 2100 Da with Telomere $\{G_3(T_2AG_3)_3\}^{4+}$ at 1672 Da. A spectrum of the cancer cells is denoted by K562; Lymphocytes cells are marked as DNA SKW; White Blood Cells are depicted as DNA WBC.

masses that are identical from the cancer (DNA-K562) to the corresponding masses of normal cells [white (DNA-WBC) and normal B-lymphocytes (DNA-SKW) blood cells] several fragments are common and identified as telomeres.

Telomere fragments were assigned to masses 1327 Da and 1672 Da on basis of prior mass analyses [11]. However, other fragments may also represent telomere codons T_2AG_3. These include fragments of various mass to charges ranging from +1 to +5. A previous mass to charge of negative ions was reported [11]; where 1327 Da corresponds to $[G_3(T_2\ AG_3)_3]^{5-}$ & 1672 Da corresponds to $[G_3(T_2\ AG_3)_3]^{4-}$. Here we observe fragments with positive (+) mass to charge mode of the mass spectrometer with signals at 1327 Da corresponding to $\{G_3(T_2\ AG_3)_3\}^{5+}$ and signal at 1672 Da corresponding to $\{G_3(T_2\ AG_3)_3\}^{4+}$. Explain why 1327 Da and 1672 Da correspond to telomere. Cancer cells in general show smaller full width half-maximum (FWHM) values, manifested as fewer molecular fragments and more isotopic differences. These smaller FWHM of cancer may stem from more isotopic clumping and more nonrandom ^{13}C &^{14}N isotopic clustering in the cancer DNA with less ^{15}N. Smaller masses tend to be nucleotides and nucleosides from the DNA while some are originated from telomeres. Medium mass pieces are larger segments of telomere domains and there are some heavier masses of telomeres themselves as well.

In Figs. 1 and 2, the mass signals are located at 1327 Da and 1672 Da. These signals are in Figs. 1 and 2 and in Supplementary Figures II & III from many different cancers cell lines include skin cancer, lymphoma, mantle B cell lymphoma & multiple myeloma. In the discussion section, we consider possible differences of these cell lines. The normal DNA is derived from several healthy volunteers.

As shown, telomere fragments have mass to charges at 1327 Da and 1672 Da for charges of 5+ and 4+, respectively. This result demonstrates that telomeres are observed in our study and as those that are reported in ref 11. However, unlike prior mass spectrometric analysis of normal DNA [11], here we have measured cancer DNA and normal DNA. Other fragments may also represent telomere codons $\{G_3(T_2AG_3)_3\}$. The prior observations also mass analyzed by negative mass to charge as it was reported that negative mass to charge observations of negative ions (4- and 5-) produce more stable fragments with greater intensities. In the current measurement, the positive mass to charge measurements (of 4+ and 5+) were made with observations of greater intensities of positive fragments from cancer DNA (possibly due to less ^{15}N) and consistent poor intensities from normal DNA. The relative difference of ratio ($^{15}N/^{14}N$) of the intensities (0.412) of the cancer and the normal telomere peaks are much larger than the standard deviation of such ratio (0.056) measured within repeated acquisitions of the MYLA2 cancer sample implying a statistical significance. Such greater

stability of cancer positive-charged DNA fragments relative to normal positive-charged DNA fragments represents a fourth difference of the cancer telomere and the normal telomere. This may be due to less 15 N in the cancer DNA. The narrower FWHMs per signals, broader distributions of signals and greater intensities are three other common characteristics of mass spectra of the cancer telomeres relative to the normal telomeres DNA, having 1) broader FWHM per signal, 2) narrower distributions of signals and 3) lower intensities of signals.

3. Discussion

Our mass to charges ratios of 1372 Da and 1672 Da are telomeric fragments measured by mass spectrometry in a positive mass to charge mode. The 1372 Da [$\{G_3(T_2\ AG_3)_3\}^{5-}$] and 1672 Da [$\{G_3(T_2\ AG_3)_3\}^{4-}$] mass to charge from reference 11 are deprotonated telomeres with charges −4 and −5, respectively. But the mass spectrometric measurements in this current study are obtained in positive mass to charge mode with 1327 Da [$\{G_3(T_2\ AG_3)_3\}^{5+}$] and 1672 Da [$\{G_3(T_2\ AG_3)_3\}^{4+}$] protonated telomere pieces having charges of +5 and + 4, respectively. The importance of 1327 Da & 1672 Da peak differences in cancer verses normal cells stems from the fact by which the ^{15}N depletion & the ^{15}N depletion in cancer which are thought to stabilize the telomere.

The mass/charge of 1327–1331 Da in negative mode from reference 11 is based on calculated formula weight of $\{G_3(T_2AG_3)_3\}^{5+}$ by polymerizeing nucleotides G, A and T by the formula with losses of OH^- 's to H_2O from the polymerizations. Such calculates a mass to charge of 1327 Da for formula $\{G_3(T_2AG_3)_3\}$ if masses of $5p^+$ are subtracted from the neutral formula weight and 5 positive charges of those protons are divided into the resulting mass. Likewise, the mass/charge for 1670 Da–1674 Da in negative mode from reference 11 is based on calculated formula weight of $\{G_3(T_2AG_3)_3\}$ by polymerizing the nucleotides G, T and A based on this formula with losses of OH^- 's from the polymerizations. The mass/charge of 1672 Da follows for $\{G_3(T_2AG_3)_3\}^{4+}$ by subtracting masses of 4 p^+ and dividing by charge 4.

In our mass spectra in positive mode, we observe the same masses for the telomere fragments at mass/charge 1327 Da and 1672 Da. But as u positive mode the fragments are protonated rather than deprotonated. So the fragments at 1327 Da and 1672 Da have charges n^+ and m^+, respectively. For 1327 Da peak, the mass of the telomere $\{G_3(T_2AG_3)_3\}$ prior to protonating is 6645.656 Da. So protonating by np^+ changes the mass to 6645.656 + n (1.007) Da. The resulting np^+ give charge of n^+. The mass to charge for the resulting $\{G_3(T_2AG_3)_3\}^{n+}$ is:

$$\{6645.656 + n\ (1.007)\ /\ n\} = 1327$$

The solution is n = 5. Therefore the 1327 Da peak has n = 5 and formula $\{G_3(T_2AG_3)_3\}^{5+}$

For the 1672 Da peak in positive mode, the mass/charge for the resulting $\{G_3(T_2AG_3)_3\}^{m+}$ is:

$$\{6645.656 + m\ (1.007)\ /\ m\} = 1672$$

The solution is m = 4. Therefore, the 1672 Da peak in our positive mass spectrometric measurement has m = 4 and the formula is $\{G_3(T_2AG_3)_3\}^{4+}$.

All four of these differing characteristics (more stable + ions, narrower FWHM, broader bands and higher intensities of cancer telomeric mass spectra relative to normal telomeric mass spectra) probably result from of different isotopic clumping of ^2D, ^{13}C, ^{15}N and ^{17}O in cancer derived telomeres versus the normal one possessing less clumping and more random distributions of these isotopes.

In general, the bonds differ in mass by 1 Da. The masses of the isotopes differences are given as delta Δm_H ($^2D - {}^1H$) = 1.0063 Da; Δm_C ($^{13}C–^{12}C$) = 1.0034 Da; Δm_N ($^{15}N–^{14}N$) = 0.9970 Da; and Δm_O ($^{17}O - {}^{16}O$) = 1.0042 Da. The mass spectra show larger peaks with mass difference $\Delta m_N = 0.997$ in both cancer and normal telomeres. See Figs. 1 and 2 and Supplimentary 1, 2, 3, and 4. A limitation of this technique is its lesser ability to measure mass difference of the $^{17}O/^{16}O$ and $^{13}C/^{12}C$ in the telomeres and DNA. The masses are more explicit and larger in cancer telomeres. This is explicit, indisputable measured quantified mass differences of ^{15}N in cancer vs normal telomeres. The cancer telomeres are enriched in ^{14}N and normal telomeres are enriched in ^{15}N. The enrichments and depletions are calculated by ratios of isotopic amounts in proportion to relative mass intensities (AU). The $^{15}N/^{14}N$ ratio is larger in normal telomere relative to cancer telomere. Of the 4 elements (H, C, N, and O); the N has larger polarity difference of its nuclear magnetic moments (NMMs) of ^{15}N having negative NMM and ^{14}N having positive NMMs. ^{12}C has zero NMM and ^{13}C has positive NMM. ^{16}O has zero NMM and ^{17}O has negative NMM. But only N has its two stable isotopes of positive and negative NMMs for more dramatic differences for greater changes in properties of N when ^{14}N is replaced by ^{15}N. The measured difference of ^{15}N in normal telomere verse cancer telomere gives evidence of prior theory of isotopes affecting stability of telomeres and isotopic changes inducing changes in unwinding of telomere and te isotope induced mechanism of cancer.

Since cancer telomeres and DNA in general have more isotopes, more fragmentations of the telomeres are induced by more negative NMMs from ^{15}N and ^{17}O isotopes, which increases the internal stabilities of the fragments (or domains) and their intensities in our data. The broader bands obtained in our system may be explained by the higher degree of clumping of these isotopes in cancer derived telomeres. In this way, the isotope clumping might affect the malignancy of the cells. The more ^{13}C in the cancer telomere with ^{14}N causes more internal stability and less fragmenting of cancer telomere. But the greater amount of ^{15}N in normal telomeres causes instability and fragmenting and unraveling of normal telomere for normal anti cancer effects and youthfulness of tissue having ^{15}N.

The FWHMs vary in the cancer fragmented DNA relative to the normal fragmented DNA. The FWHMs of the mass signals from the mass analyses are related to the isotopic positioning in the fragments as by thermodynamics and kinetics of isotopic chemistry of enrichments, redistributions, reactivities and stabilities. ^{17}O isotope in functional ^{17}OH and acetyl groups are more reactive and contributes to faster kinetic of enrichments, redistributions and depletions. The ^{13}C and ^{15}N are less labile in the methyl and amine

functional groups and more contribute to thermodynamic stabilities as the clump with ^{17}O after redistributions due to greater stabilities of ^{13}C–^{17}O and ^{13}C–^{15}N as by the positive NMM of ^{13}C contributing more binding to negative NMMs of ^{15}N and ^{17}O beyond the e^- e^- covalence in ^{13}C–^{15}N and ^{13}C–^{17}O. Normal has more random isotopes of ^{13}C, ^{15}N and ^{17}O in telomere and DNA in general for broader FWHM. Aging and cancer involve isotopic redistributions of ^{13}C, ^{15}N and ^{17}O to specific positions and bonds for isotopic clumping for causing the narrower FWHM in cancer. In case of telomeres, the ^{13}C accumulates in telomeres and ^{15}N depletes in telomeres causing the cancer telomere to less fragment and unravel and less unwind for character of cancer reproduction. By such isotopic clumping of ^{13}C certain bonds are strengthened and certain bonds are weakened for leading to specific isotopic induced cleaving of the DNA and telomeres to produce specific fragments from the cancer DNA for causing narrower FWHMs of mass fragments from cancer DNA. Specific cleaving and altered internal stabilities of cancer telomeres lead by depletion of ^{15}N to altered winding and unwinding and interactions with proteins of the cancer telomeres relative to normal telomeres for altered biology. So the isotopic enrichments of ^{13}C and depletion of ^{15}N cause telomeres to transcribe RNA and isotopic telomeres of RNA, then translate telomerase as is observed to be upregulated after cancer genesis. Telomeres in normal cells with more ^{15}N do not as easily transcribe RNA and ^{15}N enriched RNA in normal cells do not as easily translate proteins. Telomerase may have isotopes for advancing cancer. This phenomenon may also explain the stabilization of cancer telomeres.

Regarding the intensity of the telomeres derived signals, they have different intensities in cancer derived telomeres compared with normal cells derived telomeres. Laser ablation is more easily in the mass spectrometer, analogous to specific enzymes breaking non-primordial isotopes. The differential fragmentations by non-primordial isotopes between cancer and normal DNA affect the properties of these fragments.

These are general isotopic effects of ^{15}N depleting in telomeres observed in this measurement. But for different cancers there may be different isotopic patterns. But we are not certain isotopic characters of the 1 Da mass differences in all the different types of cancers. And in different types of cancers there may be in general different isotopic characters and distributions. And in the theory [4,5], even for cancer of a specific type the isotopic patterns may change for different stages of the cancer. For instance, isotopic effects causing KReb cycle, accelerated glycolysis and altered telomeric unraveling are common to all cancers and could have similar isotopic origins.

Importantly, this is not the case for macroscopic systems or in gases with two body collisions in a given reaction. In liquid and nano-domains within cells with ^{1}H, ^{14}N, and ^{31}P background, the nano-organizations by NMMs occur at normal biochemistry and biology. When unusual isotopes ^{13}C, ^{15}N, ^{17}O, ^{25}Mg and ^{33}S isotopes replace usual isotopes ^{12}C, ^{14}N, ^{17}O, ^{26}Mg and ^{32}S in this environment, they alter the collective effects of domains of the natural NMM driven processes. At lower temperatures, the nano-domain is needed for such ferro-chemistry. At higher temperatures, fewer atoms and molecules are needed for ferro-chemistry to alter the surround electronic lattices for modifying the transportations, transformations and catalysis in cellular processes such as transcription, replication and translation.

The observations described in our study are further consistent with study of isotopes in twins in space and earth [7]. The authors have found that the elongations of DNA of orbiting twin astronaut differs relative to the earth bound twin as the orbiting astronaut may experience neutrons in cosmic rays that enrich methyl groups with ^{13}C and amine groups with ^{15}N, which increases the nucleophilic attack of the DNA by $^{13}CH_3$ and $^{15}NH_2$ and thus elongating their telomeres. Cosmic neutrons may also produce ^{17}O and ^{13}C from ^{16}O and ^{12}C at driving isotopic clumping in the DNA of the astronaut in space. The $^{13}CH_3$ in the orbiting twin's telomeres may alter the degree of telomere exposure during DNA replication, as the $^{13}CH_3$ is a stronger base than $^{12}CH_3$. The water is recycled from urine of astronauts for prolong time so the stronger base $^{14}NH_3$ can strip weaker bases $^{15}NH_2$ from biomolecules to enrich $^{14}NH_2$ in amines in biomolecules and in DNA and telomeres for transforming normal cells to cancer cells in the astronauts relative to humans on surface of earth. This phenomenon may further affect DNA transcription and RNA translation.

Future studies will aim at using PDX animal models. Since the isotopic differences in cancer cells may vary, more experiments are definitely needed to elucidate this issue. Here we focused on telomere and its alterations which appear common in most cancers. Future studies may focus on other sections of DNA that appear common across many cancer cells. For instance, prior international study by pan cancer analysis of a whole genomes involving over 700 researchers observing over 2600 samples from 38 different cancer types determined 95 % of cancers caused by mutations or so-called driver mutations. In general, the researchers determined 4–5 common driver mutations shared by all the cancers. In the future, we hope to mass analyze these 4–5 mutations in DNA to see if we can observe any patterns in isotopic distributions that may cause these driver mutations.

4. Materials and method

4.1. Cell growth

The experimental system consisted of three different cell types: total white blood cells, K562 (chronic myeloid leukemia) and SKW6.4 (B lymphocyte cell line). Both cell lined were given to us by the laboratory of Prof. Pia Raanani. Total white blood cells were isolated from 5 healthy volunteers that were recruited to the study. IRB approval was: 0729-17-RMC, approved by the Rabin Medical Center IRB committee. After signing an informed consent, the blood (10 ml) was collected in a regular EDTA containing "counting" tube. Thereafter, the erythrocytes were lysed by using the Red Blood Cells lysis solution (Biological Industries, Israel) according to the provided manual. Briefly, cells were mixed with the lysis solution, agitated for 10 min and centrifuged for 2 min at 3125 RPM. The supernatant was then discarded. The lysis step was repeated twice and the pellet was used for DNA isolation.

K562 cells were cultured in the presence of RPMI-1640 growth medium containing 10 % FCS supplemented with 2 mM L-Glutamine, 100units/ml penicillin and 100 μg/ml streptomycin (Biological Industries Beit Haemek, Israel). Cells were grown in a 95 % humidity incubator with 5 % CO_2. SKW6.4 cells were cultured in the presence of RPMI-1640, 10 % FCS, 2 mM L-Glutamine and 10 mM

HEPES, 100units/ml penicillin and 100 μg/ml streptomycin (Biological Industries Beit Haemek, Israel). Cells were grown in a 95 % humidity incubator with 5 % CO_2.

4.2. DNA isolation

Cells were harvested and DNA was isolated by using the QIAamp DNA Mini Kit (Qiagen, MD, USA) according to the manufacturer's instructions. Basically, samples were first lysed using proteinase K. The lysate in buffering conditions was loaded onto the mini spin column. During centrifugation, DNA was selectively bound to the column membrane. The remaining contaminants and enzyme inhibitors were removed in two wash steps and the NA was then eluted in TE buffer.

Towards assessing DNA from normal donors, the blood was collected from donors that signed an informed consent. IRB approval was: 0729-17-RMC, approved by the Rabin Medical Center IRB committee.

4.3. MALDI

DNA samples of K562, SKW, and WBC were analyzed. 20 μL of water were added to each sample tube to dissolve the dried samples. Sample water solution was mixed with THAP (2′,4′,6′-Trihydroxyacetophenone monohydrate) matrix (saturated in 25 mM ammonium citrate in ACN/Water 50/50) at 1:3 (v/v) sample to matrix ratio. The mass measurements were performed on a Bruker Rapiflex MALDI TOF/TOF mass spectrometer (Bruker Daltonics, U.S.A.) in positive linear mode and with a 355 nm Bruker scanning smartbeam™ 3D laser.

We have combined a detailed description of the method in the revised manuscript as follows:

Initially, DNA samples of K562, SKW, and WBC were analyzed. 20 μL of water were added to each sample tube to dissolve the dried samples. Subsequently DNA of more cancer cells were analyzed, including Acute Monocyte Leukemia (THP1); Breast Carcinoma (MDA231); Breast Carcinoma (MCF7); Mantle BCell Lymphoma (Z138); Mantle BCell Lymphoma (JECO-1); Mantle BCell Lymphoma (REC A2); Mantle BCell Lymphoma (REC A1); Skin Lymphoma (MYLA 1); Skin Lymphoma (MYLA 2); T Lymphoblast (JURCAT 2); and T Lymphoblast (JURCAT1). Additional DNA of different normal cells was also analyzed: Normal 1, Normal2, Normal 3, and Normal 4. Sample solutions of each were mixed with 20 μL of water in sample tubes. Each sample was then mixed with THAP (2′,4′,6′-Trihydroxyacetophenone monohydrate) in 25 mM ammonium citrate in ACN/Water at 50/50 ration by volume to form matrix. The resulting samples were injected into mass spectrometer. The mass measurements were performed on a Bruker Rapiflex MALDI TOF/TOF mass spectrometer (Bruker Daltonics, U.S.A.) in positive linear mode and with a 355 nm Bruker scanning smartbeam™ 3D laser. The spectra were measured with ion source voltage of 20 kV. No background gas was used for the analyses. The spectra were measured in vacuum with pressure of 4.0 e−7 mbar.

Telomere fragments from masses of telomere are divided by charges as we have stated the isotopic mass to charge for 1327 Da and its formula and the mass to charge for 1672 Da and its formula. We have corrected the formulas to have charges 5+ and 4+.

5. Conclusions

The isolation of DNA from different normal cells and many cancer cells of different type results in various patterns of decompositions of the cancer DNA and normal DNA as they are ablated in the laser of a mass spectrometer. Many patterns of the ablated cancer DNA fragments similar; many patterns from the ablated normal DNA are similar. The difference between the two is in functionalization, de-functionalization, intensity, broadness and heaviness of signal bands, full width half maxima and isotopic contents. These differences probably stem from isotopic differences of $^{13}C/^{12}C$, $^{2}D/^{1}H$, $^{15}N/^{14}N$, $^{17}O/^{16}O$, $^{25}Mg/^{24}Mg$, and $^{33}S/^{32}S$. On the basis of that model [4,5] the different, nonzero nuclear magnetic moments of these non-primordial uncommon stable isotopes (^{2}D, ^{13}C, ^{15}N, ^{17}O, ^{25}Mg and ^{33}S) relative to the primordial common stable isotopes (^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{24}Mg and ^{32}S) induce difference internal multi-body chemical dynamics and enzymatic resulting in DNA synthesis affecting the malignant character of that DNA. Similar differences in other biological processes related to transcriptions, translations and other biological biochemistry might also be affected by that phenomenon.

Additionally, this work may explain recent experiments conducted with twin by NASA [7]. In this study, the telomere length of prolonged, space orbiting twin brother were elongated relatively to those of the earth bound twin brother. According to our theory [4,5], cosmic rays may transmute ^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{24}Mg, ^{32}S, and/or ^{42}Ca to ^{2}D, ^{13}C, ^{15}N, ^{17}O, ^{25}Mg, and/or ^{43}Ca, thus affecting telomerase activity and telomere dynamics of the twin brothers' DNA in orbit relative to those brother on the surface of earth. Our data may further theoretically explain the observed nonrandom common multiple mutations for oncogenesis as observed among variety of many cancers *in vivo* [3]. Another phenomenon that our study might explain is the observed gravitational selective killing of cancer cells relative to normal cells [8]. In addition, our data is consistent with observations regarding higher extent of nonrandom methylations of DNA in cancer cells [9] versus that of normal cells and [10]. Our data further point to the importance of isotopic induced acetylations as leading to the massive mutations in DNA as well as histones resulting in oncogenesis.

Data availability statement

There is no research data except for the published one.

CRediT authorship contribution statement

Reginald B. Little: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Maiya Snowton:** Methodology, Investigation, Data curation. **Orit Uziel:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23519.

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