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In silico Study of Effects of Polymorphisms on Biophysical Chemical Properties of Oxidized N-Terminal Domain of X-Ray Cross-Complementing Group 1 Protein

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Abstract—Base excision repair (BER) is the major pathway involved in removal of endogenous and mutagen-induced DNA damage. The X-ray cross-complementing group 1 protein (XRCC1), which participates in BER, is a scaffolding protein. The oxidized XRCC1 N-terminal domain (NTD) forms additional interactions with DNA polymerase β (Pol β). Any change in the residues of a protein (XRCC1, XRCC4, etc.) may alter its stability and function. Many coding regions of genes have single nucleotide polymorphisms (SNPs) that change the conformation of their products, and they are probably involved in some diseases. The R7L and R107H mutations are located in the XRCC1-NTD. In the present study, biophysical chemical properties of oxidized XRCC1-NTD (wild type or mutants) were investigated at different temperatures (290, 295, 298, 301, 304, 309, 310, 311, and 312 K) in water using *in silico* molecular mechanic computational methods. Comparison of the average calculated potential energies of oxidized XRCC1-NTD reveals that the R7L mutation increases stability, but the R107H and R7L&R107H mutations are destabilizing. Therefore, mutant types of this protein (R107H or R7L&R107H) may not function correctly. Furthermore, quantitative structure–activity relationship (QSAR) of oxidized XRCC1-NTD and docking assay showed that the R7L mutation is advantageous but the R107H and R7L&R107H mutations are disadvantageous for XRCC1-NTD, and in the latter cases it cannot interact with Pol β as well as the wild type does. Hence, DNA repair may be defective. Also, using the equation $dE = \partial E/(\partial T)_V \cdot dT + \partial E/(\partial V)_T \cdot dV$, it was determined that the best temperature for normal activity of oxidized XRCC1-NTD is exactly the natural body temperature (310 K).

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The most common forms of DNA damage, particularly under normal physiological conditions (i.e. in the absence of environmental challenges), are base modifications, abasic (AP) sites, and thousands of DNA single-strand breaks (SSBs) in cells each day [1, 2]. If not repaired, such genetic damage poses a threat to both genetic stability and cell survival, resulting in an increased frequency of mutations and chromosome aberrations [3-8]. Base excision repair (BER) is the major pathway for removal of endogenous and mutagen-induced DNA damage. This repair process involves several sequential reactions. A damage-specific DNA glycosylase recognizes and removes a damaged base by cleavage of the N-glycosylic bond connecting the base to the sugar-phosphate backbone. The resulting apurinic/apyrimidinic site (AP site) is recognized by AP endonuclease 1 (APE1), which incises the phosphodiester bond 5′ to the AP site, followed by addition of one nucleotide to the 3′-OH end of the incised AP site and excision of the base-free sugar phosphate residue by DNA polymerase β (Pol β). Finally, DNA ligase completes the repair by sealing the DNA ends (reviewed in [9]).

The X-ray cross-complementing group 1 protein (XRCC1) is a single strand break DNA repair protein that is found in eukaryotic species ranging from insects to humans. It is composed of an N-terminal domain (NTD), a central, breast cancer susceptibility protein-1 C-terminal (BRCT) domain (BRCT-I), and a C-termi-

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nal BRCT domain (BRCT-II) [10-13]. XRCC1 has no known enzymatic activity, but it provides multiple functions in BER, which include assembly of DNA repair complexes containing Pol β, poly(ADP-ribose) polymerase (PARP), and DNA ligase III (reviewed in [14]). It is thought that by binding Pol β and damaged DNA, XRCC1 may protect the template strand at the site of DNA damage so that short patch repair can proceed efficiently [15].

Two crystal forms of the XRCC1-NTD contacting Pol β have been solved, and this has revealed that the XRCC1-NTD is able to adopt a redox-dependent alternate fold characterized by a disulfide bond, and substantial variations in secondary structure, folding topology, and electrostatic surface. Although most of these structural changes occur distal to the interface, the oxidized XRCC1-NTD forms additional interactions with Pol β, enhancing affinity by an order of magnitude [16, 17]. Phenotype studies have demonstrated that early embryonic lethality is associated with knockout of the mouse XRCC1 [18]. Rodent cells lacking XRCC1 are hypersensitive to a broad range of gene toxins [19-22]. Loss of XRCC1 also results in decreased genetic stability, including increased frequencies of spontaneous and/or induced chromosome translocations and deletions [21-23].

The stability of a protein is easily affected by single amino acid substitutions that change one or more of the following: a hydrogen bond, a salt bridge, a hydrophobic interaction, the volume of the residue, a disulfide bond, or the relative position of two aromatic rings [24]. The gene-coding region of *XRCC1* has several non-conservative sites: R399Q, R194W, R280H, R7L, and R107H [25]. Therefore, these single nucleotide polymorphisms (SNPs) can alter the conformational stability of XRCC1 and thus the binding or regulatory activities, and hence alter the efficacy of XRCC1 in DNA repair [26]. Only the R7L and R107H mutations are located in the XRCC1-NTD (residues 1-161), and for the other mentioned SNPs there is, unfortunately, no existing domain with a PDB entry code. However R7L and R107H can change the stability and activities of the XRCC1-NTD. Hence, using computational methods the stability and behavior of the wild type XRCC1-NTD (Arg7 and Arg107) and mutated type XRCC1-NTDs (Leu7 and His107 alone and together) were investigated. So far, several experimental studies have been performed to investigate the relation between of R399Q, R194W, and R280H SNPs with some cancers. The authors of those investigations mentioned that direct or inverse relations between them may come from changes in protein conformation [27-34], but there has been no investigation that has studied this issue. Therefore, for the first time, in the present study, this subject has been investigated using computational methods, and the results predict which SNPs will be advantageous or disadvantageous for a person. We also know temperature changes can alter protein structure and stability. Hence, the effects of temperature were investigated as well.

MATERIALS AND METHODS

The X-ray structure of the wild type oxidized XRCC1-NTD was obtained from the Protein Data Bank (PDB). The PDB entry code of the NTD is 3K77. Molecular modeling and computation were performed using the HyperChem 8.1.81 software [35]. Using this software, the molecular mechanic (MM) method, Monte Carlo (MC) simulation, and amber 99 force field was used for computation of potential energy for any type of oxidized NTD, wild type or R7L, R107H, and R7L&R107H mutants, at various temperatures (290, 295, 298, 301, 304, 309, 310, 311, and 312 K) in the presence of water. The mutations (R7L and R107H) were modeled with the Swiss PDB Viewer 4.04 PC software. Geometry optimization of the oxidized NTD (wild type and mutants) was implemented before computation of single point or Monte Carlo simulation. We also calculated the volume and surface quantitative structure–activity relationship (QSAR) properties of the oxidized NTDs (wild type and mutants) after the Monte Carlo run at all studied temperatures in the presence of water. Furthermore, docking of the domain (wild type and mutants) with the Pol β catalytic domain (Pol β CD) was investigated using the AutoDock Vina software [36].

The equation $dE = \partial E/(\partial T)_V \cdot dT + \partial E/(\partial V)_T \cdot dV$ of the wild type oxidized XRCC1-NTD was determined by computation of partial derivatives of potential energy (∂E) and partial derivatives of volume (∂V) at different temperatures (E is the potential energy of the protein, V is its volume, and T is the temperature of the system). $\partial E = E_{max} - E_n$, $T_{Emax} - T_n$. $E = f(T, V)$, and $V = g(T)$; therefore, $\partial E/\partial V = \partial E/\partial T \times \partial T/\partial V = (\partial E/\partial T)/(\partial V/\partial T)$.

The values of $\partial E/\partial T$ and $\partial V/\partial T$ at any temperature can be calculated from the dependences shown in Figs. 4 and 5 (see further).

Paired sample *t*-test analyses were performed using the Statistical Product and Service Solutions (SPSS) v.20.0 software.

RESULTS AND DISCUSSION

Our study shows the difference of potential energy of the wild type oxidized N-terminal domain of XRCC1 and its mutant types (R7L, R107H, and R7L&R107H) at different temperatures in the presence of water (Fig. 1). We know that water is critical not only for the correct folding of proteins, but also for the maintenance of their structures [37]. Under conditions of sufficient hydration, proteins can attain their active minimum-energy conformation (native). Water molecules can lubricate the move-

Potential energy (PE), volume (V), and surface (S) (QSAR properties) of oxidized XRCC1-NTD SNPs

SNPs	Average ± S.D.			Comparison of the averages (p value)								
	PE, kcal/mol	V, Å^3	S, Å^2	wild with R7L			wild with R107H			wild with R7L&R107H		
				PE	V	S	PE	V	S	PE	V	S
Wild	−2155.30 ± 1370.55	30134.63 ± 30.64	7727.55 ± 21.08	0.254	0.000	0.000	0.496	0.009	0.007	0.541	0.000	0.000
R7L	−2866.46 ± 860.103	29979.33 ± 17.91	7638.34 ± 22.78									
R107H	−1473.00 ± 1921.38	30084.23 ± 15.13	7709.31 ± 7.60									
R7L&R107H	−2077.41 ± 557.521	29997.59 ± 38.45	7688.78 ± 20.78									

ment of the amino acid backbone and side groups by rapid formation and exchange of hydrogen bonds. Generally, even if the temperature of the system is increased somewhat, the movement of the amino acid backbone and side groups will increase. Thus, this changes some intramolecular interactions and interactions between water and protein, and the protein conformation can change somewhat (denaturation). Also, thermal denaturation is linked to the breakup of the 2-D-spanning water network around the protein (due to increasing hydrogen bond breakage with increasing temperature) [38]. Consequently a given protein has a particular potential energy at any given temperature, and this energy is determined by its amino acid composition. The Lennard–Jones (LJ) function of non-bonded terms of molecular mechanics potential energy functions includes a weakly attractive component at long distances (the van der Waals energy) and a strongly repulsive component at

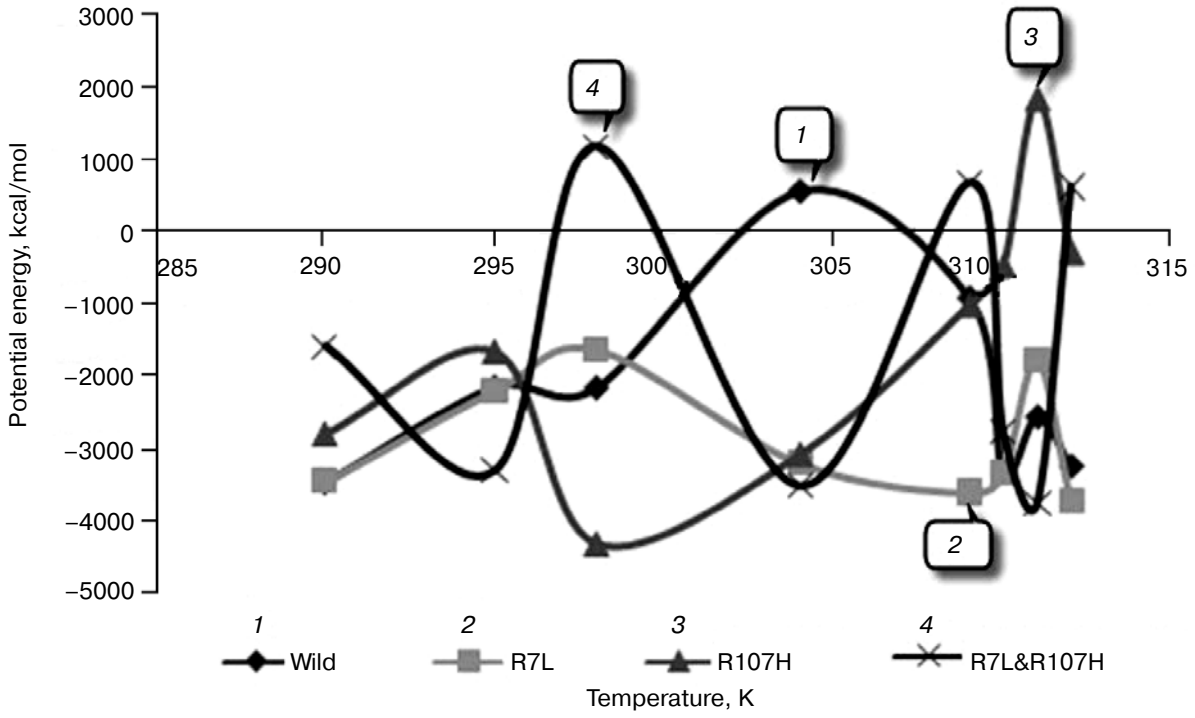

Fig. 1. Comparison of potential energy of wild type oxidized XRCC1-NTD (*1*) or mutant oxidized XRCC1-NTD (R7L) (*2*), mutant oxidized XRCC1-NTD (R107H) (*3*), and mutant oxidized XRCC1-NTD (R7L&R107H) (*4*) at different temperatures in the presence of water.

short distances. The repulsive component is sensitive to small atomic displacements: the LJ energy of a protein crystal structure can decrease by hundreds of kcal/mol even upon local energy minimization in spite of only slight changes in the atomic coordinates [39]. We know that amino acid alterations affect not only the mutation site, but also other parts of the protein far from the site, although the structural changes are not large [40, 41]. Therefore, the potential energy of a given protein can change even on single amino acid substitutions, as the overall results of the present study confirm.

The table presents the average of potential energy and standard deviation for oxidized XRCC1-NTD wild type and R7L, R107H, and R7L&R107H mutants in the presence of water at different temperatures from 290 to 312K. When the average potential energy of the wild type is compared with the mutants R7L, R107H, and R7L&R107H, we will see that all *p* values are insignificant, but with consideration of Fig. 1 the difference between energies at some temperatures is dramatic, for example, at 298K the difference between the wild type and R7L&R107H or between R7L and R107H is about 2500 kcal/mol, and the difference between wild type and R7L at 300K is about 1000 kcal/mol, which is very large and significant.

As shown in Fig. 1, the potential energy level generally decreases if Arg7 (Fig. 1, curve *1*) changes to Leu (Fig. 1, curve *2*) in the presence of water, but as mentioned before the difference of the average of potential energy is not significant. Therefore the stability of the NTD will increase slightly and this will positively affect the interaction with Pol β. Figure 2 (see color insert drawn using SPDBV and VMD software) shows that Arg7 occurs in the β-sheet structure of the XRCC1-NTD, and it is an exposed residue. Because the mean propensities of Arg and Leu for β-strand conformations are 0.85 and 1.15, respectively [42], and the positively charged Arg7 exists on N-terminal side of XRCC1-NTD (with negative electrostatic potential) (Fig. 2a), then if Arg7 is changed to Leu (Leu has a hydrophobic side chain) the NTD will become more stable. Furthermore, we know that protein folding is driven by hydrophobic interactions, due to the unfavorable entropy decrease forming a large surface area of nonpolar groups contacting water [43, 44]. Leucine contains a rather nonpolar and flexible side chain that is ideally suited for packing in the protein interior [45]. The enthalpy of transfer of nonpolar groups from the protein interior into water is negative below about 25°C (298K) and positive at higher temperatures (and *vice versa*) [46]. Therefore, by changing Arg7 (Arg has hydrophobicity index −19.9 kcal/mol [47]) to Leu (its hydrophobicity index is 2.28 kcal/mol [47]) and by increasing the temperature from about 298K, the side group of Leu may move from the exterior into the interior of the protein, hence the conditions are better for protein folding. Thus, the potential energy of the protein structure of the mutant decreases, and it becomes more stable.

Figure 1 also shows that above 310K, the R107H mutant (Fig. 1, curve *3*) has much more potential energy than the wild type, and below this temperature, specifically at 298K, it has less potential energy. Thus, the substitution of His with Arg107 above body temperature has negative effect on the stability of oxidized XRCC1-NTD. Figure 2 shows that Arg107 is located in turn elements of the NTD and is a buried residue. The side chain of buried Arg107 hydrogen bonds with Phe93 (oxygen of the carbonyl group) (Fig. 2b). When Arg107 is changed to His, using SPDBV software and calculating H-bonds indicates that His does not form a hydrogen bond, so the NTD becomes unstable. As mentioned above, by transferring a nonpolar group from the protein interior into water the enthalpy is negative below about 25°C and positive above that temperature [46]. Hence, by exchange of the buried Arg107 to His, whose hydropathy index is −3.2 (more than Arg), at low temperature the protein becomes more stable, but at higher temperatures when the side group of His can completely move from the interior of the protein into water (brought about by protein denaturation) the protein becomes more unstable. Indeed, the behavior of the XRCC1-NTD when Arg107 is changed to His is almost the opposite of the case with substitution of Leu with Arg7.

Also, Fig. 1 makes it obvious that the existence of the R7L&R107H mutant in the NTD has a nearly neutral influence decreasing the stability of the domain.

According to the discussion given above, the existence of the R107H and/or R7L&R107H mutations in the oxidized XRCC1-NTD at some temperatures causes the NTD to become unstable; consequently, it cannot function properly in BER. Genomic and proteomic instability are proposed to be factors in tumor initiation; hence, when a protein does not perform its task (i.e. XRCC1 in DNA repair) it can be a reason for some problems and diseases in humans [48]. Some studies have shown that genetic polymorphisms of *XRCC1* are associated with colorectal cancer, lung cancer, glioma, and cervical cancer [27-34], and in the present investigation it was confirmed that some SNPs (R107H and R7L&R107H) demonstrate the instability of oxidized XRCC1-NTD, and as a result these SNPs can be related with some diseases including some cancers. However, the present study shows that the R7L mutation further stabilizes the oxidized XRCC1-NTD, and the existence of this SNP causes the NTD to function better than the wild type.

The QSAR properties (average of volume and surface) of the wild type and mutant XRCC1-NTDs after a Monte Carlo run at any temperature in the presence of water are shown in the table. On the basis of the *p* values in the table, the average of the volume and surface of the XRCC1-NTD with the R7L SNP is significantly greater than the wild type. Thus, the R7L mutant can interact with Pol β better than the wild type, and consequently the R7L SNP is an advantage for humans, and it may be

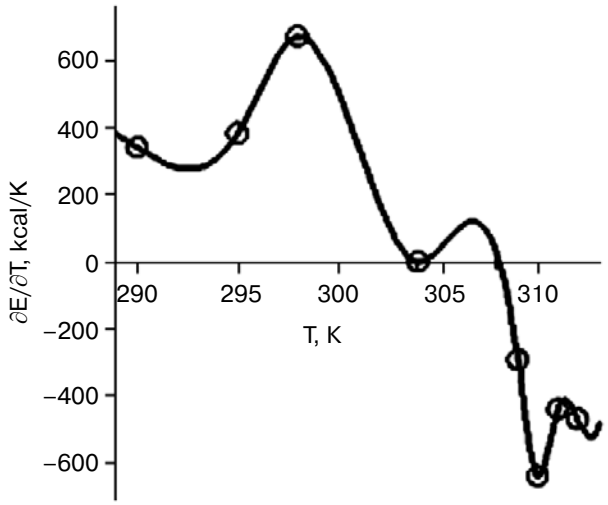

Fig. 4. Change in $\partial E/\partial T$ versus temperature (T) for wild type oxidized XRCC1-NTD. The equation of this curve is: $F(y) = -0.006321625736\ y^7 + 13.39733381\ y^6 + 1.470361376 \cdot 10^{15} - 12166.94879\ y^5 - 3.401967329 \cdot 10^{13}\ y + 6.137942218 \cdot 10^6\ y^4 + 3.372943505 \cdot 10^{11}\ y^2 - 1.857656080 \cdot 10^9\ y^3$.

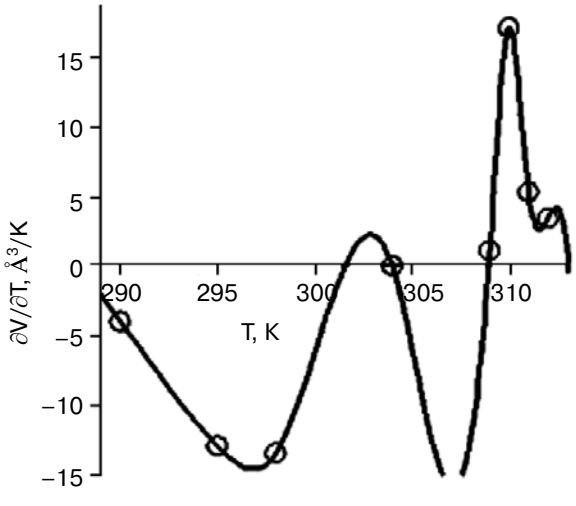

Fig. 5. Change in $\partial V/\partial T$ versus temperature (T) for wild type oxidized XRCC1-NTD. The equation of this curve is: $F(y) = 0.0003133589284\ y^7 - 0.6640774589\ y^6 - 7.286853342 \cdot 10^{13} + 603.0714662\ y^5 + 1.686013138 \cdot 10^{12}\ y - 3.042259880 \cdot 10^5\ y^4 - 1.671684969 \cdot 10^{10}\ y^2 + 9.207143607 \cdot 10^7\ y^3$.

involved in DNA repair more actively. On the contrary, the averages of volume and surface of the XRCC1-NTDs with the R107H or R7L&R107H SNPs are significantly lower than the wild type. As a result, the XRCC1-NTD that contains the R107H or R7L&R107H SNPs cannot interact with Pol β properly. Therefore, the R107H and R7L&R107H SNPs are disadvantageous for humans, and these XRCC1-NTDs may not be able to function adequately in DNA repair.

The docking of XRCC1-NTDs (residues 1-161) as ligands with Pol β CD (residues 91-338) as the receptor was accomplished using the AutoDock Vina software [36]. Matthew et al. [49] indicated that both the oxidized and reduced forms of XRCC1-NTD bind specifically to the thumb subdomain of Pol β CD (Fig. 3; see color insert). Therefore, in the present study the XRCC1-NTD was docked with the thumb subdomain of Pol β CD. The best docking of the XRCC1-NTD to Pol β CD is shown in Fig. 3. The results of the docking study indicate that the binding affinity of the wild type oxidized XRCC1-NTD in the best mode is 695.9 kcal/mol, and the binding affinity of R7L, R107H, and R7L&R107H SNPs are 699.1, 662.3, and 670.45 kcal/mol, respectively. We know a high affinity ligand has a much greater tendency to bind to the receptor [50]. On this basis, the R7L SNP has more binding affinity than the wild type, and the binding affinity of R107H and R7L&R107H SNPs are less than the wild type. When the binding affinity of the XRCC1-NTD to Pol β CD is low, it certainly could not perform its function well.

We also investigated docking of XRCC1-NTD/Pol β CD as a receptor with DNA (a 20-bp deoxyoligonucleotide with PDB entry code: 3GBI has a single strand break) as a ligand (Fig. 3b). As seen in Fig. 3a, there is a groove between the XRCC1-NTD and the Pol β CD; thus, after performing the docking it was shown that the best mode for binding of DNA is at this location (Fig. 3b). It was determined that the binding affinity in the best mode for the wild type NTD is 175.2 kcal/mol, while for R7L, R107H, and R7L&R107H SNPs it is 178.0, 172.0, and 173.5 kcal/mol, respectively. According to these results, which are similar to those mentioned above, we conclude that the binding affinity of the R7L SNP is slightly greater than the wild type, and the binding affinity of the R107H and R7L&R107H SNPs are slightly less than the wild type. Although Arg7 and Arg107 are distal to the interface of the XRCC1-NTD and Pol β CD, the R7L SNP forms higher interaction with the Pol β CD, while R107H and R7L&R107H SNPs have lower interactions. Finally, the structural effects of these SNPs are indeed low but not zero. The overall conclusion of this investigation is that the R7L SNP in XRCC1-NTD is useful, but the R107H and R7L&R107H SNPs are disadvantageous for BER.

Curves of $\partial E/\partial T$ and $\partial V/\partial T$ versus temperature (T) and their equations for the wild type obtained using the Maple software are shown in Figs. 4 and 5. In the equations presented in the legends to Figs. 4 and 5, $F(y)$ is $\partial E/\partial T$ and $\partial V/\partial T$, respectively, and y is T. Therefore, at a specific temperature we can exactly calculate how much the potential energy and volume changes are. These figures show that changes in $\partial E/\partial T$ and $\partial V/\partial T$ in the range of natural body temperature (310K or 37°C) are more than at other temperatures. Thus, the best temperature

for functioning of the XRCC1-NTD and probably for other dynamic proteins in humans is 37 ± 0.5°C. Therefore, increase in body temperature is dangerous for health, and moreover proteins cannot act in their normal manner. Indeed, this can be a reason for many diseases including cancers.

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