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RESEARCH ARTICLE

Do Gamma Rays of Cancer Radiotherapy Effect on the Sequence of Collagenase Gene?

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Abstract

Cancer is a genome disease, initiated by mutations in genes that usually control cell growth and division. Matrix metalloproteinases (MMPs) have important roles in both of tumor invasion and spread. The present study was conducted to evaluate the effect of ionizing radiation (in the level of treatment doses of γ-rays). The action of collagenase gene was examined *in vivo* by using a model of solid tumor carcinoma (EAC). Eighty male mice were used for this experiment; it is divided into 8 groups, 10 mice / each; which were Normal, beside three groups of normal mice and three groups of tumor transplanted mice each of them exposed to the following accumulated doses (6, 9 and12 Gy). Whole body mice were exposed to gamma radiation from Cobalt-60 (^{60}Co) twice a week for three weeks with different doses after 12 days of injected ehirlich tumor in the right thigh region. The different doses of ionizing radiation exposure resulting in different changes (Mutations) on the sequence of collagenase gene in normal and malignant implanted groups and the level of these mutations were height in the malignant groups.

Introduction:-

The malignant transformation of cells is collectively determined by six essential alterations (commonly shared by all types of human tumors) to cell physiology: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Each of these physiologic changes represents the successful breaching of an anticancer defense mechanism hardwired into cells and tissues, (Hanahan and Weinberg, 2000). Mutations in several critical genes can lead to tumors (Loeb et al., 2003).

Metastasis is the spread of cancer cells from the primary tumor to the new metastatic sites via blood or lymph vessels (Stacker et al., 2002). Metastasis is a highly inefficient but a deadly process (Sakari and Joan, 2013). It was long thought that some tumor cells acquire new mutations not initially present in the primary tumor cells making them metastatic. Later on, it was suggested that metastatic cells have an intrinsic signature pattern, the ‘‘poor-prognosis signature’’ that accounts for their metastatic behavior. Recent studies have merged these metastasis theories by showing that in addition to a poor-prognosis signature, the metastatic cells must activate additional genes, which are not activated in the primary tumor (Kang et al., 2003).

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Although no single gene has been identified as a major regulator of metastasis in all tumors, many animal models indicate a critical role for the MMPs, including MMP-2 and MMP-9. For example, gene expression analysis of human tumors has linked MMP-9 with a poor prognosis in breast cancer (van't Veer et al., 2002).

Radiation therapy is the treatment of cancer with ionizing radiation. Radiation works by damaging the DNA (genetic material) within the tumor cells, making them unable to divide and grow. Radiation is often given with the intent of destroying the tumor and curing the disease. However, although radiation is directed at the tumor, it is inevitable that the normal, non-cancerous tissues surrounding the tumor will also be affected by the radiation and therefore damaged, (Burnet et al., 1996).

External Beam Radiation Therapy (EBRT) creates a radiation beam and aims it at the tumor. The radiation adequately covers the tumor but minimizes the dose to the non-tumor normal tissues. Radiation is given in fractions rather than as a single dose, and the use of this fractionated radiotherapy allows normal cells time to repair between each radiation session, protecting them from injury. A number of different radiotherapy schedules have been suggested to overcome this problem, (Shah et al., 2000).

These include hyper fractionation, in which the time between fractions is reduced from 24 hours to 6 or 8 hours to enhance the toxic effects on tumor cells, (Fu et al., 2000), while still preserving an adequate time interval for the recovery of normal cells. Continuous hyper fractionated accelerated radiation therapy (CHART) is an intense schedule of treatment, in which multiple daily fractions are administered within a short period of time. Clinical studies have shown benefits of altered fractionation over conventional treatment for several cancers, including head and neck cancer (Goodchild et al., 1999) and inoperable lung cancer (Kate et al., 2014).
MMP family has extended to a group of at least 23 zinc-bound proteinases, which are involved in remodeling of the ECM, (Visse and Nagase, 2003).

MMP implicated in many physiological processes including embryonic development, organogenesis, wound healing processes and bone remodeling (Page-McCaw et al.,2007).

(Hu et al., 2007 ; Chabottaux and Noel, 2007) indicated that MMPs are involved in many pathological conditions like inflammation and the invasion of cancer cells by extracellular matrix (ECM) remodeling, including degradation of the basement membrane.

The role of MMPs in different stages of tumor development has been studied, showing up-regulation of various MMPs in colorectal cancer (CRC), (Zucker and Vacirca, 2004).
The collagenases consist of MMP-1, MMP-8, MMP-13 and MMP-14. Their ECM substrates include different collagens and the collagenases are activated by several other MMPs and plasmin.MMP-13, which can be activated by MMP-3, (Chakraborti et al., 2003), seems to have a central role in the MMP activation cascade (Leeman et al., 2002), activating both MMP-2 and MMP-9.

MMP-13 expression is low in normal epithelial cells but strongly up-regulated in tumor cells, inflammatory cells and occasionally fibroblasts, (Chiang et al., 2006; Vizoso et al., 2007). MMP-13 has a role in angiogenesis, (Zijlstra et al., 2004), and is associated with poor survival in CRC patients (Leeman et al., 2002), and several other cancers, (Nina Johansson, et al., 1999, Culhaci et al., 2004; Chiang et al., 2006).

Material and Methods:-

Animals: Male Swiss mice weighted range of 16-20 g were left 2, weeks on commercial diet and tap water for adaptation before the beginning of the experiment under optimum conditions of light and humidity.
Mice were infected subcutaneously (at right thigh region) with 1 X10 single cell/ml (0.2 ml) submission isolated from Ehrlich ascites carcinomas.

Whole body animals were exposed to accumulated doses of γ- rays (1, 1.5 and 2 Gy) twice a week for 3, weeks from Cobalt-60 (^{60}Co) Cell, located at Middle Eastern Regional Radioisotope Center for the Arab Countries, (Dokki, Giza, Egypt). The dose rate of cell = 1Gy / 3, min. in October 2013.
The present study was carried out on adult male mice and was categorized into 8 groups, (10 mice/each) as shown in the following:

N group served as normal; T.C (Tumor control) group of implanted with Ehrlich tumor; R 1.0 Gy group exposed to 1.0 Gy γ-rays; R 1.5 Gy group exposed to 1.5 Gy of γ-rays; R 2.0 Gy group exposed to 2.0 Gy of γ-rays; T+R 1.0 Gy group implanted with Ehrlich tumor and exposed to 1.0 Gy of γ-rays; T+R 1.5 Gy group implanted with Ehrlich tumor and exposed to 1.5 Gy of γ-rays and T+R 2.0 Gy group implanted with Ehrlich tumor and exposed to 2.0 Gy of γ-rays.

Molecular techniques:-

DNA Extraction: Total cellular DNA was isolated from tissue samples of infected right thigh of the mice, according to manufacturer protocol of Omega Co. (USA. LMt.), (Mohamed and M. Yacout, 2014).

Polymerase chain reaction (PCR) for gene detection and amplification: *Taq* DNA polymerase was purchased from Sigma-Aldrich Chemie GmbH, Germany (#D9307).*Taq* Green master mix was used for collagenase IV gene amplification of all groups according to the manufacture protocol.

PCR purification: Gene JET PCR purification kit (Thermo Scientific; # K0702, USA) was used for DNA purification from the agarose gel according to the manufacture protocol. Gene JET PCR purification kit effectively removes primers, $dNTP_S$, unincorporated labeled nucleotides, enzymes, and salts from PCR and other reaction mixtures. The collagenase IV gene primer pair used here included the forward primer F (5′- CTTGCCTAGCAGAGCCCATT-3′) and the reverse primer R (5′- AGTGGTCAGCCAAGGGAAAG-3′).

DNA Sequencing: ABI PRISM®3100 Genetic Analyzer was applied to the products of the PCR purification procedure. The DNA sequencing was performed by Macrogen In. Seoul, Korea. A common platform for fluorescent dideoxy DNA sequencing is the capillary-based automated DNA sequencer. Capillary-based sequencing uses liquid self-coating polymers to separate DNA fragments and has vastly improved the efficiency of DNA sequencing. Data was obtained using the Big Dye® Terminator 3.1 Sequencing Standard. POP-4 performance Optimized Polymers were used as the separation matrices. A 10× Genetic Analysis buffer with EDTA, diluted to 1×, was used as the running buffer for POP-4. 3730 Buffer (10×) with EDTA, diluted to 1×, was used for POP-7 runs. All reagents, standards, software programs, and updates were from Applied Biosystems (Foster City, CA, USA) (Detwiler et al., 2004). Sequence data were collected using ABI PRISM® 3100Data Collection™ v3.0, and Data were analyzed with ABI PRISM®3100 DNA Sequencing Analysis Software™ v5.1.

Molecular analysis (Bioinformatics) tools:-

T coffee is a multiple sequence alignment online tool. It was used to compare all sequences in normal and treated groups to detect mutations which appear as single nucleotide polymorphism (http://tcoffee.crg.cat/apps/tcoffee/do:regular) (Notredame *et al*. 2000). Geneious 4.8 (Trülzsch et al., 2007); Biomatters Ltd; http://www. Geneious.com/) was used for the Phylogenetic analysis by the Neighbor Joining method. RNA2 prediction 2.0 (Brodsky et al., 1995), Genebee.msu.ru/services/rna2_reduced.html) was used for the prediction of RNA secondary structure by free energy minimization method.

Results:-

PCR and sequencing techniques were exploited to find out the mutations in specific portion of collagenase IV gene of the eight different group's mice. DNA ladder represented in the 1st lane was separated into bands with different lengths. Whereas, the PCR products of collagenase IV gene for the different normal and other groups can be seen as a single band in each lane of approximately the same length 219 base pairs (bp). **N,** T.C, R 1.0 Gy, R 1.5 Gy, R 2.0 Gy, T+R 1.0 Gy, T+R 1.5 and T+R 2.0 Gy groups were arranged respectively in lanes 1 to 8 (Fig.1).

The targeted bands were isolated from the gel, and then PCR products were purified from the agarose gel and were sequenced. The resulted collagenase IV (partial) consensus sequences of all studied groups were shown in (Fig.1). The sequences of collagenase IV gene in all groups were of 219 nucleotides.

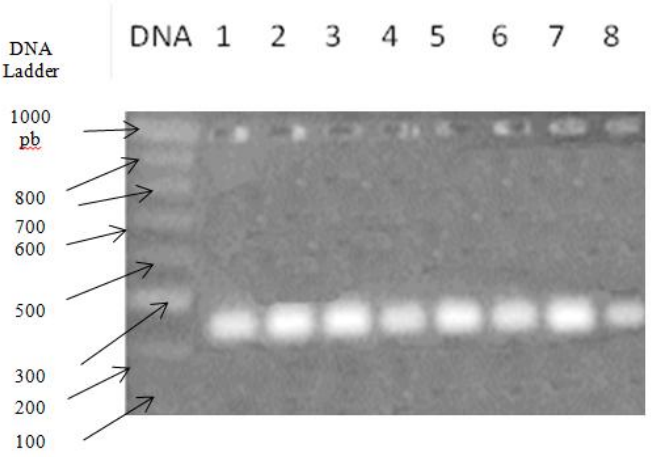

Figure 1: PCR products of collagenase IV gene with approximately 219 pb.

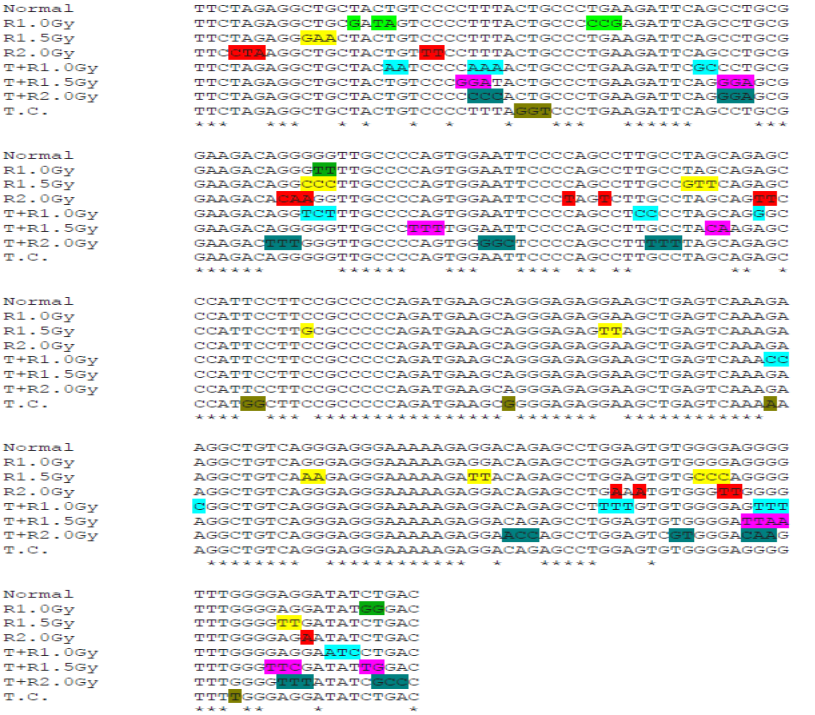

Figure 2: T coffee alignment of the collagenase IV DNA consensus sequence of all studied groups.

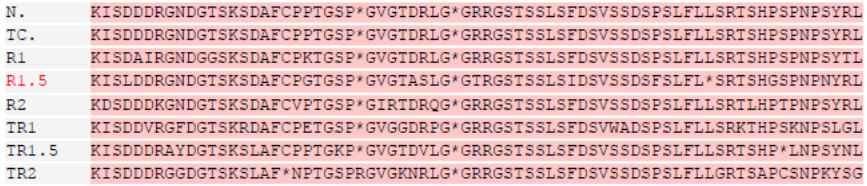

Figure 3: The prediction of protein translated from normal and studied groups of collagenase IV sequence

The online software T coffee was used to build multiple sequence alignment (MSA) of the DNA and of the protein amino acids. MSA of the DNA for collagenase IV gene was shown in figure (2). And, MSA of amino acids of collagenase IV gene was shown in figure (3). TC group collagenase IV gene sequence was different from the normal. There were some single nucleotide polymorphisms (SNPs) obtained when TC group compared to the normal, such as; C/G, T/G, G/T, T/G, C/G, A/G, G/A and G/T at positions 28, 29, 30, 105, 106, 127, 149 and 204, respectively. All SNPs, of TC when compared to the normal, did not change the amino acids of the protein due to giving silent mutations (SM) at positions; 10, 35, 36, 43, 50 and 68, respectively.

MSA obtained some SNPs when R 1.0 Gy group compared to the normal. Some nucleotides changed in R1.0Gy group, such as; T/G, C/T, T/A, T/C, G/C, A/G, G/T, G/T, C/G and T/G at positions; 14, 16, 17, 34, 35, 36, 61, 62, 215 and 216, respectively. The SNPs of the nucleotides and its position changed the most of amino acid arrangement in R1.0Gy group when compared to the normal, such as; D/A, D/I, T/G, P/K and R/T at positions; 5, 6, 12, 21 and 72, respectively.

MSA showed some nucleotide changes in R1.5Gy group when compared to the normal collagenase IV gene sequence, such; C/G, T/A, G/A, G/C, G/C, G/C, T/G, A/T, G/T,C/G, G/T, A/T, G/A, G/A, G/T, G/T, G/C, G/C, G/C, A/T and G/T at positions; 10, 11, 12, 60, 61, 62, 92, 93, 94, 110, 135, 136, 160, 161, 174, 175, 193, 194, 195, 208 and 209, respectively. These nucleotide changes changed and rearranged the amino acids of the protein which produced from R1.5Gy group, such; D/L, SM, P/G, D/A, R/S, R/T, SM, F/I, P/F, SM, Stop Codon (SC), P/G and S/N at positions; 4, 20, 21, 31, 32, 37, 45, 46, 54, 58, 59, 65 and 70 respectively.

Also, MSA clarified some changes in the sequence of R2.0Gy group when compared to the sequence of normal collagenase IV gene, such; T/C, A/T, G/A, C/T, C/T, G/C, G/A, G/A, C/T, C/T, A/T, G/T, G/A, G/A, G/T, A/T and G/A at positions; 4, 5, 6, 20, 21, 58, 59, 60, 82, 85, 98, 99, 186, 188, 195, 196 and 210, respectively. The amino acids, of the protein which expressed from the R2.0Gy group, changed and rearranged according to the changes in the nucleotides and its positions, such; I/D, R/K, P/V, V/I, G/R, L/Q, SM, S/L, SM, S/T and SM at positions; 2, 7, 20, 28, 29, 33, 62, 63, 65, 66 and 70, respectively.

The alignment of collagenase IV gene sequence of T+R1.0Gy group when compared to the normal sequence produced some SNPs, such as; T/A, G/A, T/A, T/A, T/A, A/G, G/C, G/T, G/C, G/T, T/C, G/C, A/G, G/C, A/C, A/C, G/T, G/T, A/T, G/T, G/T, G/T, T/A, A/T and T/C at positions; 17, 18, 24, 25, 26, 43, 44, 60, 61, 62, 88, 89, 98, 149, 150, 151, 185, 186, 187, 198, 199, 200, 212, 213 and 214, respectively. The SNPs of the sequence changed the protein amino acids of T+R1.0Gy group when compared to the normal, as; D/V, SM, N/F, S/R, SM, P/E, T/G, L/P, S/W, S/A, T/K, S/T, SM, P/K, Y/L and R/G at positions; 6, 8, 9, 15, 20, 21, 30, 33, 50, 51, 62, 63, 66, 67, 71 and 72, respectively.

When the sequence of T+R1.5Gy collagenase IV gene group aligned to the normal gene, some SNPs appeared, such; C/G, T/G, T/A, C/G, C/G, T/A, C/T, A/T, G/T, G/C, C/A, G/T, G/T, G/A, G/A, G/T, A/T, G/C, T/A, A/T and T/C at positions; 23, 24, 25, 45, 46, 47, 69, 70, 71, 94, 95, 197, 198, 199, 200, 207, 208, 209, 212, 213 and 214, respectively. Also, due to the nucleotide changes, the amino acids changed, such; G/A, N/Y, SM, D/L, SM, S/K, R/V, SC, P/L, SM, SM, SM and R/N at positions; 8, 9, 15, 16, 23, 24, 32, 66, 67, 69, 70, 71 and 72, respectively.
MSA of the sequence alignment of T+R2.0Gy group when compared to the normal gave some SNPs, as; T/C, T/C, T/C, C/G, C/G, T/A, A/T, G/T, G/T, A/G, A/G, T/C, G/T, C/T, C/T, C/A, A/C, G/C, G/C, T/G, G/T, G/C, G/A, G/A, A/T, G/T, G/T, T/G, G/C and A/C at positions; 24, 25, 26, 45, 46, 47, 57, 58, 59, 75, 76, 77, 89, 90, 91, 177, 178, 179, 190, 191, 192, 197, 198, 199, 208, 209, 210, 216, 217 and 218, respectively. The amino acids of T+R2.0Gy group changed in types and its positions, as; SM, N/G, SM, D/L, SC, P/N, SM, EXTRA- R, T/K, D/N, SM, S/G, H/A, S/C, P/S, S/K, R/S and L/G at positions; 8, 9, 15, 16, 19, 20, 25, 26, 30, 31, 59, 60, 64, 66, 67, 70, 72 and 73, respectively.

RNA is a single strand so it tends to form complicated base-pairing interactions due to its increased ability to form hydrogen bonds. Regions with base pairing are called stems and regions with single strand are called loops. Stems need more energy to break the hydrogen bonds. The RNA secondary structure was assessed by genebee service (http://www.genebee.msu.su/services/rna2_reduced.html).

Figures (4), (5), (6), (7), (8), (9), (10) and (11) showed the RNA secondary structure prediction of collagenase gene consensus RNA sequences in all studied groups.

The RNA secondary structure of normal, TC, R1.0Gy and T+R1.5Gy groups of collagenase IV gene had eight stems and of free energy equaled -40.1, -45.8, -35.9 and -45.0 Kkal / mol., respectively. On the other hand, the RNA secondary structure of R1.5Gy and R2.0Gy groups had nine stems and of free energy equaled -33.6 and -45.1 Kkal/ mol., respectively. The RNA secondary structure of collagenase IV gene for T+R1.0Gy group sequence had seven stems with free energy equaled -36.5 Kkal/ mol; however T+R2.0Gy group had ten stems with free energy of -62.3 Kkal/ mol.

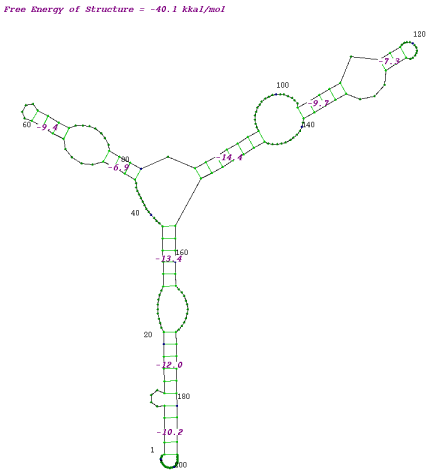

Fig. 4: The predicted structure of RNA in normal group, with free energy of structure = -40.1 kkal/mol.

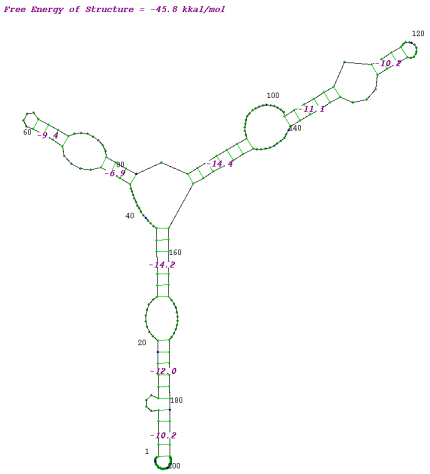

Figure 5: The predicted structure of RNA in tumor control group, with free energy of structure = -45.8 kkal/mol.

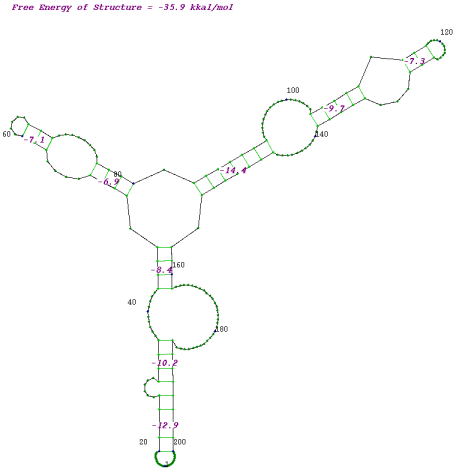

Figure 6: The predicted structure of RNA in irradiated 1.0 Gy group, with free energy of structure = -35.9 kkal/mol.

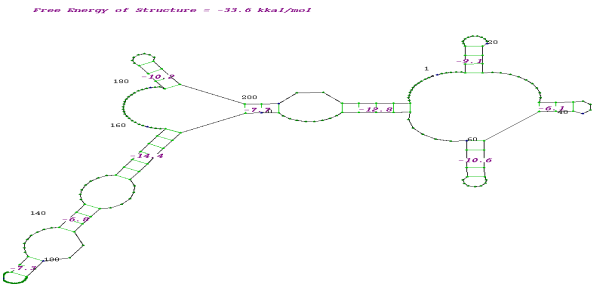

Figure 7: The predicted structure of RNA in irradiated 1.5 Gy group, with free energy of structure = -33.6 kkal/mol.

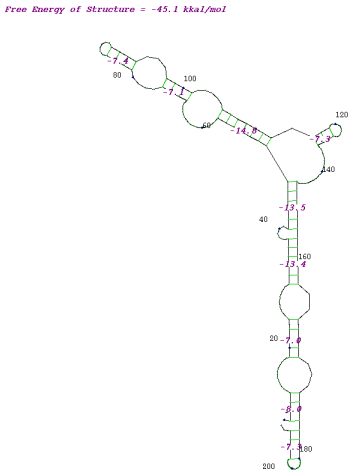

Figure 8: The predicted structure of RNA in irradiated 2.0 Gy group, with free energy of structure = -45.1 kkal/mol.

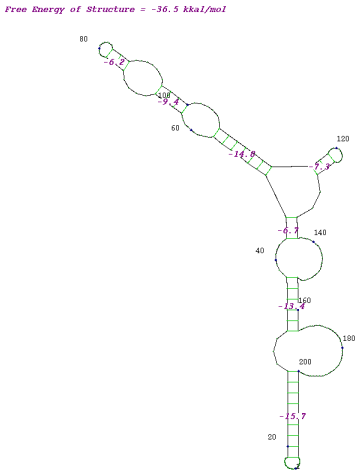

Figure 9: The predicted structure of RNA in tumor + 1.0 gray group, with free energy of structure = -36.5 kkal/mol.

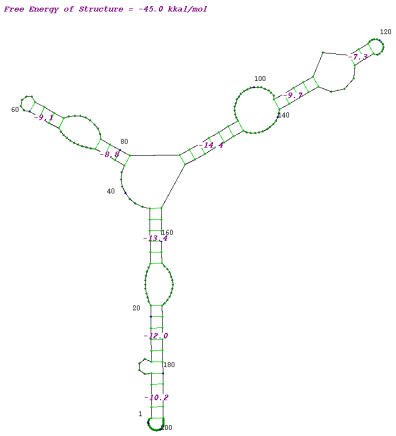

Figure 10: The predicted structure of RNA in tumor + 1.5 gray group, with free energy of structure = -45.0 kkal/mol.

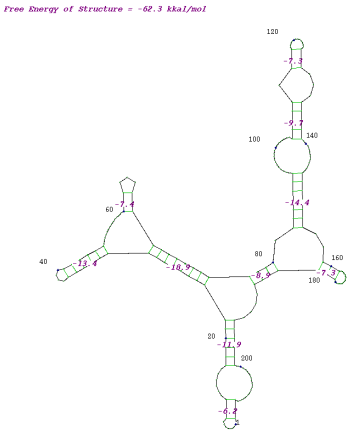

Figure 11: The predicted structure of RNA in tumor + 2.0 gray group, with free energy of structure = -62.3 kkal/mol.

Discussion:-

Genome-wide association studies (GWAS) have provided an excellent platform in follow up of identifying common risk variants implicit many polygenic diseases and radiation effects (Pennisi, 2010).

Metastasis of cancer takes place in a sequence of linked steps which require multiple host-tumor interactions. The initial event, local invasion, requires the tumor cells to penetrate the epithelial basement membrane and enter the underlying stroma, a process which involves attachment to the membrane, secretion of proteinases which degrade the extracellular matrix components, and migration through the preprepared area of proteolysis (Stracke et al., 1992).

Metalloproteinases which degrade type IV collagen are known to be involved in the invasion metastasis process; for example, the capacity of rat mammary carcinoma cell sub lines to metastasize is related to their level of type IV collagenolytic activity (liu and Rose, 1995 ;Kang et al., 2003).

Controlled degradation of extracellular matrix (ECM) is an important feature in a variety of biological processes, such as embryonic development, tissue remodeling and tissue repair. On the other hand, ECM is an essential part of growth, invasion, and metastasis of malignant tumors. Matrix metalloproteinases (MMPs) are a family of extracellular zinc-dependent neutral endopeptidases collectively capable of degrading essentially all ECM components and they play an important role in ECM remodeling in physiologic situations, such as embryonal development, tissue regeneration, and wound repair. In addition, distinct MMPs play important, and sometimes opposite roles at different steps of tumor growth, invasion, and metastasis, and recent observations suggest that MMPs also play a role in cancer cell survival (Overall and López-Otín, 2002; Egeblad and Werb, 2002).

It has been known, that proteolytic enzymes are associated with the malignant phenotype and involved in tumor invasion in vitro and in vivo experimental models and human neoplasms (Tryggvason et al., 1987; Mignatti and Rifkin, 1993; Nagase, 1998). However, at the early stages of invasion and tumor genesis the roles of distinct proteinases are still unclear. Use of the experimental systems of fibroblast transformation may assist in the elucidation of proteinase role in complex process of carcinogenesis.

Radiation therapy is one of the most broadly used therapies for cancer, acting on a wide array of tumor types. More than 25% of adults in developed countries will develop a malignant tumor, and nearly half of these patients are likely to be treated with some form of radiation therapy (Harrison et al., 2002). Radiation was the first effective adjuvant treatment for cancer, and numerous improvements over the past several decades have made it a mainstay of oncology. However, the effects of radiation are often transient, stimulating intensive searches for agents that can synergize with it (Chmura et al., 2001; McGinn and Lawrence, 2001; Wachsberger et al., 2003). In addition, ionizing radiation was reported to cause oxidation of the sulfhydryl groups to the corresponding dithiols and induce conformational changes of membrane proteins (Birben et al., 2012).

Gamma radiation affects biological membrane in different ways. To study and monitor the effects of radiation one needs a series of analyses to explore the different damaging events that may occur. Free radicals formed during irradiation can cause a variety of membrane changes including lipid peroxidation, hydrolysis of phospholipids head groups, lipid-lipid crosslinks, disulfide bridge formation and amino acid residue damage in membrane proteins and lipid-protein crosslinks (Nabila et al., 2009).

Multiple sequence alignment (MSA) is an important initial step when deriving the most common biological models, including phylogenetic reconstruction, structural homology modeling, and functional inference through domain profile comparisons. It is one of the most challenging tasks in computational biology for its very high computational complexity. One can detect the most reliable portions from the sequenced data to be studied (Chang et al., 2014; Pramanik et al., 2014). So, wetargeted collagenase IV gene from the sequenced data. Sequence similarity searches against biological sequence databases using the algorithm computational tools have become one of the most used bioinformatics approaches (Lee et al., 2012; Neuman et al., 2013).
The Central Dogma of biology defined three categories and an information flow in which DNA stores the information, and RNA carries the information to proteins that then carry out all biological functions (Herschlag et al., 2015). So, it was appropriate to use the genebee program to predict the RNA and the translated protein sequences for further analyses.

Alignments are processed for Single Nucleotide Polymorphisms (SNPs) and indels detection. SNP is a single base in the DNA differs from the usual base at that position. SNPs are the marker of choice in genetic analysis and also useful in locating genes associated with mutations. Indels, another type of DNA mutations, are inserted or deleted pieces of DNA (Seal et al., 2014; Narzisi and Schatz, 2015). These mutations caused the genetic variations; however SNPs constitute the larger compartment in this variation (Ma and Lu, 2011). Our data distinguished that, there were a lot of missing, extra addition and substitution of nucleotides in collagenase IV gene of all groups. These mutations could affect the predicted protein's amino acid sequences of collagenase IV gene of these groups.

The single-stranded nature of RNA provides the plasticity needed for it to fold into diverse secondary structures (e.g., hairpins or three-way junctions) and tertiary structures (e.g., pseudoknots and G-quadruplexes) that govern its functional roles (Kwok *et al*., 2015). RNA hairpins consist of a double-stranded RNA stem, often containing and a terminal loop. They can guide RNA folding, determine interactions in a ribosome, protect messenger RNA (mRNA) from degradation, serve as a recognition motif for RNA binding proteins or act as a substrate for enzymatic reactions. RNA hairpins formed in different positions within different types of RNAs; they differ in the length of the stem, the size of the loop, the number and size of bulges, and in the actual nucleotide sequence (Svoboda and Cara, 2006).

The classical RNA secondary structure models, derived from RNA sequences, consider the Watson–Crick A/U and G/C base pairs as well as the G/U wobble pair (zuSiederdissen et al., 2011). Many computational algorithms for predicting RNA secondary structure models are based on empirical free energy parameters determined from thermodynamic data of small RNA model compounds (Schuster, 2006).
Thermodynamics is a major determinant of secondary structure prediction and thus of evolution of structured RNAs, as the free energy minimization alone typically predicts correctly about 70% of secondary structure. However for

more accuracy experiments should be carried out to mapping RNA structure (Mathews, 2014). The models resulted from the free energy minimization could be improved by applying the statistical mechanics of RNA folding, predicting pseudoknots, and using homologous sequences (Mathews and Turner, 2006).

Many factors were found to regulate the protein expression. These are chromatin structure, transcriptional initiation, processing and modification of mRNA transcripts, Transport of mRNA into cytoplasm, stability or decay of mRNA transcripts, initiation and elongation of mRNA, posttranslational modification, and intracellular transport and degradation of the expressed protein (Adeli, 2011).

In this study the RNA secondary structure was built by the online gene bee services. The results revealed that the number of the stems and the free energy calculated for RNA secondary structures predicted from consensus RNA sequence transcribed from collagenase IV gene in R1.5Gy, R2.0Gy, T+R1.5Gy and T+R2.0Gy groups were slightly different than that of the normal, TC, R1.0Gy and T+R1.0Gy sequences. Also, the RNA secondary structure stability in these studied groups will be different. Consequently the rate of the protein translation for R1.0Gy, R1.5Gy and T+R1.0Gy studied groups will be slightly greater than that of the normal and the other groups sequences, but it could be nonfunctional, due its free energies. Whereas, the free energy of TC, R2.0Gy, T+R1.5Gy and T+R2.0Gy group's secondary structure was greater than the free energy of the normal. Then, the secondary structure of TC, R2.0Gy, T+R1.5Gy and T+R2.0Gy groups would be much more stable than the normal and the other groups. Also, the rate of TC, R2.0Gy, T+R1.5Gy and T+R2.0Gy groups' protein production would be much more less than the protein production of normal, and nonfunctional.

RNA secondary structure affected protein translation rate with many ways. The unwinding of every structure in mRNA by the ribosome decreases the elongation rate of the protein (Mao et al., 2014). Also, in vivo translation of thymidylate synthase mRNA the expression was greatly influenced by the GC content of the 5' coding region (Pedersen-Lane et al., 1997). Moreover, the translational efficiency of collagenase IV was inversely correlated with the stability of the mRNA secondary structure, the presence of base-pairing in the consensus Kozaki sequence, the number of start codons in the 5' - UTRs, and the length of the 5' -UTRs (McClelland et al., 2009).

In the present study, the molecular observations and bioinformatics results showed that, there were different mutations in the IV collagenase gene depending on the dose of γ- rays and tumor implanted. These mutations were less in normal groups which exposed to radiation (3 different doses) than implanted groups with tumor when exposed to the same doses of radiation. These mutations lead to changes in their resulting RNA secondary structure, so the minimum free energy and number of stems were changed. According to these changes the ribosomal RNA translation rate changed and the needed energy to translate into protein and the resulting protein could be changed. So, the resulting structure and function of hormones and enzymes could be affected.

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