Review

Relationship between Exposure to Low Dose of x-ray and DNA Hypomethylation in Solid Tumors and Hematological Malignancies*

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X-rays are used in diagnostic and interventional radiology, and for treatment of certain benign and malignant diseases. Although these procedures utilize only low doses of radiation, the long term effects of exposure to these radiations are not known. Transfer of radiation energy to the cells results in DNA damage, mutations, chromosomal instability, and apoptosis in a dose-dependent manner. When organisms are exposed to ionizing radiation (IR), radiation energy is transferred to the cellular biomolecules, by which they are ionized or excited. This can have adverse effects, like breaks in chemical bonds, production of reactive oxygen species (ROS), and cross-linking of biomolecules, all of which lead to cellular damage. Approximately 60%–70% of these damages are mediated by ROS generated by the radiolysis of water, and remaining 30%–40% are through direct radiation[1]. When cells are exposed to IR, the resultant ROS interact with DNA, causing strand breaks, base oxidation and cross-linking. Formation of 8-hydroxydeoxyguanosine (8-OHdG) is the most common oxidative DNA damage, with a high mutagenic potential. Cells have antioxidant defense mechanisms to neutralize the damaging effects of ROS. Oxidative stress is a state in which ROS production exceeds the capacity antioxidant defense.

8-OHdG produced by ROS, binds to adenine instead of cytosine during the replication and leads to GC-->TA transversion mutation. This damage is repaired by the base excision repair (BER) system, in which, 8-oxo-deoxyguanine DNA glycosylase 1 (OGG1) specifically recognizes and excises 8-OHdG[2], and this is released into the bloodstream and is excreted through the urine. Increasing evidence suggest that 8-OHdG is involved in the formation of DNA-protein cross-links, and inhibits replication, transcription, and repair[3]. 8-OHdG causes the double-strand breaks in telomeres, leading to their fragmentations and irreversible truncation[4]. The double-strand breaks arise due to excision of 8-OHdG by OGG1 during DNA repair. 8-OHdG has been used as a biomarker of oxidative stress. In the last decade, investigations suggested that 8-OHdG may have some biological functions, as summarized by Marmiy and Esipov[5]. It is believed that 8-OHdG plays a role in regulating gene expression[6], controlling inflammatory and autoimmune reactions[7], and activating antioxidant systems. Presence of 8-OHdG on the promoter regions may start the gene expression. It was shown that presence of 8-OHdG in G-quadruplexes in promoters of proto-oncogenes *c-Kit* and *c-Myc* destabilizes the structure of these quadruplexes, and transforms them into the duplexes, which makes their transcription easier[8]. 8-OHdG in the gene promoters decrease the methylation of these sites, leading to activation of transcription[9].

For a long time, radiation was thought to damage only the targeted areas during irradiation. Over time, the bystander effect was identified[10], which is described as the effects of radiation seen in off target, non-irradiated cells. Bystander effects arise from short-range communication of radiation-induced stress signals through the gap junctions between cells, secretion of ROS, cytokines, and growth factors released from irradiated cells into the bloodstream. These molecules in turn induce ROS and DNA damage, dysregulate epigenetic mechanisms and gene expression in neighboring unirradiated tissues[11]. Low doses of IR induce ROS, but its level may not be enough to cause mutations[12]. However, ROS can modulate gene expression in non-target tissues via epigenetic dysregulation and induce cellular transformations, which can lead to tumor

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development.

EPIGENETICS

Epigenetic alterations are to heritable changes in gene expression that occur without alteration in DNA sequence. Epigenetic modifications activate or inactivate the genome at specific times and locations to yield particular cellular phenotypes. Epigenetic regulatory mechanisms include DNA methylation, histone modifications, and regulation through non-coding RNA. DNA methylation is the first discovered and best-studied epigenetic modification. It is essential for normal development in mammals and is associated with several key processes, including genomic imprinting, X-chromosome inactivation, repression of transposable elements, aging, and carcinogenesis[13]. There are two basic types of normal methylation in eukaryotic cells. First is de novo methylation, involved in the rearrangement of the methylation patterns during embryogenesis, and the second is associated with the differentiation processes in adult cells[14].

DNA methylation involves covalent addition of a methyl group to the fifth carbon of cytosine, forming 5-methylcytosine (5-mC), mostly located at cytosine phosphate-guanine sites (CpG islands) in the 5'-untranslated regions of gene promoters. DNA methylation is catalyzed by DNA methyltransferases (DNMTs), and the methyl group donor is S-adenosyl-L-methionine (SAM). Promoter hypermethylation causes gene silencing *via* two ways. 1) Methylated DNA prevents the binding of transcription factors to the gene promoter, and 2) methylated regions of the DNA are occupied by methyl-CpG-binding domain proteins (MeCPs) which bring together other epigenetic components, forming compact and inactive heterochromatin[15]. On the contrary, hypomethylation of the promoter enhances the transcription of the gene. Histone methylation also takes part in the regulation of gene expression. It can either increase or decrease the transcription of genes, depending on the amino acids (lysine, arginine, or histidine) that are methylated, and the number of methyl groups attached.

IR-INDUCED DNA HYPOMETHYLATION AND CARCINOGENESIS

Aberrant DNA methylation refers to widespread demethylation and site-specific gene hypermethylation. Demethylation affects CpG dinucleotides in both repetitive elements and in the CpG islands located in gene-specific promoters. Long interspersed nuclear element 1 (LINE-1) and Alu elements belong to a family of non-long terminal repeat retrotransposons, comprising almost 30% of the human genome. Alterations in the DNA methylation status of these repetitive elements often lead to their activation and retrotransposition. LINE-1 are heavily methylated in normal human tissues, and hypomethylated in many types of tumors[16]. Both genome-wide hypomethylation and gene-specific promoter hypermethylation are frequently detected in cancer. Demethylation of repetitive or transposable elements, or across the genome, leads to genomic instability, increased rate of mutagenesis and development of cancer[17]. Alterations in DNA methylation pattern at CpG islands in promoters may cause aberrant expression of the genes involved in cell proliferation and differentiation, giving rise to tumor. Hypomethylation of the promoters of oncogenes results in their upregulation and over-production of the oncoproteins. In healthy cells, promoters of tumor-suppressor genes have a methylation pattern that allows their expression, to regulate apoptosis and cell survival. Their hypermethylation is an important step in the inactivation of tumor suppressor genes, which may lead to cancer[18]. Aberrant DNA methylation seen in cancer-related genes is presented in Table 1[19-34].

Exposure to low dose of IR promotes changes in both global and gene-specific promoter methylation. In experimental animal models, both direct and bystander exposure to radiation showed dose-dependent, sex- and tissue-specific effects on DNA methylation patterns[35]. A single dose of radiation is not enough to cause a significant long- or short-term changes in global DNA methylation patterns[36]. However, chronic exposure to even low doses of IR is a more potent inducer of epigenetic effects and a more effective genome destabilizer[35]. Increased global DNA methylation was observed in human B lymphoblast cell line HMy2 as an adaptive response to long term exposure to low dose of IR. Global DNA hypermethylation was accompanied by increases in DNMT1 and MeCP2 expression, and heterochromatin formation in these cells[37]. On the other hand, Wang et al.[38] detected global as well as specific hypermethylation in 811 regions of genome in mice after chronic whole body exposure to low dose of IR. Genomic hypomethylation, detected in blood 2 h post-irradiation, was associated with downregulation of DNMT1 and MeCP2 in a tissue-specific manner, but was not retained at one month

post-irradiation. Hypermethylated regions were more prevalent in gene involved in DNA repair, cell cycle, apoptosis, hippo signaling pathway, GTP catabolic process, and intracellular transport[38]. One

Table 1. Aberrant DNA methylation in cancer-related genes

Gene	Function	Tumor type	References
APC	Tumor suppressor	Breast,	[19]
BIM	Pro apoptotic/tumor suppressor in B cell	Leukemia	[20]
BRCA1	DNA repair	Breast	[21]
CDH1	Cellular adhesion regulator/tumor suppressor	Thyroid	[22]
CDKN 2A (p16)	Cyclin-dependent kinase inhibitor/tumor suppressor	Lung, leukemia, brain	[23-25]
DAP kinase	Promotion of apoptosis	Thyroid	[22]
DBC1/BRINP1	Cell cycle arrest/tumor suppressor	Leukemia	[24]
DKK3	Tumor suppressor	Leukemia	[24]
DPPA2	Oncogene	Thyroid	[26]
EGFR	Cell differentiation and proliferation	Breast	[27]
GSTP1	Detoxification	Breast	[28]
hMLH 1	DNA repair	Thyroid	[29]
INSL4	Oncogene	Thyroid	[26]
MGEA1	Tumor-specific antigen	Brain	[25]
NOTCH4	Oncogene	Thyroid	[26]
O^6MGMT	DNA repair	Leukemia, brain	[24,25]
PTEN	Tumor suppressor	Breast, lung, thyroid, brain	[22,23,25,30]
RARβ2	Retinoic acid receptor	Breast, thyroid	[22,31]
RASSF1A	Cell cycle control	Breast, thyroid	[22,32]
RB	Cell cycle control	Brain	[25]
SEPT9	Cell division and migration	Breast	[33]
SHOX2	Transcriptional regulation	Lung	[23]
SLC5A8	Sodium-coupled monocarboxylate transporter	Thyroid	[22]
SOX17	Transcriptional regulation	Breast, lung, thyroid	[22,23,34]
TCF21	Transcriptional regulation	Lung	[23]
TCL1B	Oncogen	Thyroid	[26]
TIMP3	Metalloproteinase inhibitor/tumor suppressor	Thyroid	[22]
TP53	Tumor suppressor	Brain	[25]
TSHR	TSH receptor gene	Thyroid	[22]

Note. *APC*: Adenomatous polyposis coli, *BIM*: BH3-only protein, *BRCA1*: Breast cancer 1, *CDH1*:Cadherin 1, *CDKN2A*: Cyclin-dependent kinase inhibitor 2A, DAP-kinase: Death-associated protein kinase 1, *DBC1/BRINP1*: Deleted in Breast Cancer-1/bone morphogenetic protein/retinoic acid inducible neural-specific, *DKK3*: dickkopf WNT signaling pathway inhibitor 3, *DPPA2*: Developmental pluripotency associated 2, *EGFR*: Epidermal growth factor receptor, *GSTP1*: Glutathione S-transferase P, *hMLH1*: Human MutL homolog 1, *INSL4*: Insulin like 4, *MGEA1*: melanoma-associated antigen 1, *NOTCH4*: Notch Receptor 4, *O^6-MGMT*: O^6-methylguanine DNA methyltransferase, *PTEN*: Phosphatase and tensin homolog, *RARβ2*: Retinoic acid receptor beta 2, *RASSF1A*: Ras association domain family 1 isoform A, *RB*: Retinoblastoma, *SEPT9*: Septin 9, *SHOX2*: Short stature homeobox 2, *SLC5A8*: Solute carrier family 5 Member 8, *SOX17*: Sex related region Y HMG-box 17, *TCF21*: Transcriptional regulation, *TCL1B*: T Cell Leukemia/Lymphoma 1B, *TIMP3*: Tissue inhibitor of metalloproteinases, *TP53*: Tumor protein P53, *TSHR*: Thyroid stimulating hormone receptor.

of the hypermethylated genes reported in this study was *Rad23b*, involved in DNA damage recognition and repair. Product of another hypermethylated gene reported in this study, DNA-damage-inducible transcript 3 (Ddit3), plays a key role in the regulation of cell cycle by inhibiting the G1 to S phase transition. Interestingly, both these genes displayed tissue-specific hypermethylation. This study suggested that promoter hypermethylation, rather than global hypomethylation, is more stable, and able to dysregulate the expression of important genes and influence IR-induced pathogenesis. IR-induced methylation changes in the genome are shown in Table 2.

Alterations in DNA methylation patterns may not be isolated events, but may also be associated with histone methylation. Trimethylations at histone H3 lysine 9 (H3K9), histone H3 lysine 27 (H3K27) and histone H4 lysine 20 (H4K20) are responsible for the formation of transcriptionally silent heterochromatin structure. Tumor cells show some characteristic changes in histone methylation. Recent studies revealed that exposure to IR, even at low doses, alters histone methylation status. For example, histone H3K9 and histone H4K20 trimethylation are regulated negatively after exposure to both low- and high-dose of IR[39].

Taken together, aberrant DNA methylation in cancer-related genes is a common feature of tumors. Exposure to low dose of IR promotes aberrant DNA methylation, and chronic exposure is an effective inducer of changes in DNA methylation. This may be the missing link between medical radiation exposure and cancer development.

X-RAY EXPOSURE DUE TO MEDICAL IMAGING AND CANCER RISK

Exposure to x-rays in diagnostic procedures constitutes a significant part of the annual radiation exposure from all sources. Although doses of single procedures are low in standard radiographic examinations, patients who may need repeated examinations to follow their cardiac, urinary, pulmonary, orthopedic conditions, may receive relatively high cumulative doses. Specific doses from various medical imaging techniques are shown in Table 3[40-43]. Computed tomography (CT) examinations use radiations in a narrower range but have relatively higher average effective doses. Cancer risk is reported to be higher in individuals who have undergone repeated CT examinations as compared with age-matched controls[44]. Exposure of the breast tissues to radiation during the chest CT scanning has critical importance, especially in girls and young women. Increased use of CT in pediatrics has enhanced the concerns about cancer risk, because children are more sensitive to the carcinogenic effects of IR and have many years of life for cancer development[45]. The lifetime risks of cancer that may be attributed to childhood CT scans is shown in Table 4[46-54].

The coronary angiography and percutaneous coronary interventions are essential for the diagnosis and treatment of ischemic heart diseases. It was reported that low dose of IR used in cardiac imaging increases the risk of cancer in patients without a history of cancer[55].

Radiation therapy for benign diseases such as tinea capitis, enlarged tonsils, thymus, and thyroid gland was more common in the past. Although only moderate doses were used to treat various benign diseases, radiation-related tumors in or near the irradiated area have been reported. Treatment with radioiodine-131 is thought to have no side effects, but there is some evidence showing an increased risk for breast, kidney, and stomach cancers due to radioiodine-131 treatment[56].

Radiation therapy, solely or combined with other therapeutic approaches (surgery or chemotherapy), is used in treating many types of cancer. The goal of radiotherapy is to destroy tumor cells with a lethal

Table 2. IR-induced methylation changes in genome

Gene/Location	Function	References
Alu	Repetitive DNA elements	[16]
Ddit 3 gene	Cell cycle arrest	[38]
H3K9	Histone protein	[38]
H4K20	Histone protein	[38]
LINE-1	Repetitive DNA elements	[16]
Rad23b gene	DNA Repair	[38]

Table 3. Typical effective dose values for both CT and non-CT imaging examinations

Item	Typical effective dose value (mSv)
CT	
Abdomen-pelvis	8
Angiogram aorta (chest, abdomen, pelvis-rule out dissection or aneurysm)	24
Angiogram of thorax (rule out pulmonary embolism)	15
Chest	5.1
Chest (pulmonary embolism)	10
Head	2.3
Lower limbs (excluding pelvis)	0.6
Lung V/Q scan	2.2
Multiphase abdomen and pelvis	31.0
Neck	2.2
Sinuses	0.6
Spine	6
Trauma CT 'pan scan' (head, neck, chest, abdomen pelvis)	34
Radiography	
Abdomen radiograph (anteroposterior)	1.8
Cervical spine radiograph	0.2
Chest radiograph (PA and lateral)	0.1
Extremities radiograph	0.005
Knee radiograph	0.005
Hip radiograph	0.7
Lumbar spine radiograph	0.6
Pelvis radiograph	0.6
Shoulder radiograph	0.01
Thoracic spine radiograph	1.0
Other	
Abdomen angiography	20
Barium swallow	1.5
bone densitometry (DEXA)	0.001
Bone scan (nucleer)	6.3
Cardiac perfusion (sestamibi)	12.5
Cardiac resting ventriculography	7.8
Coronary angiography	5–15
Diagnostic cardiac catheterization	7
Lung ventilation/perfusion	2.0
Mammography	0.7
Myocardial perfusion imaging	15.6
Percutaneous coronary intervention	15
Upper GI series	6

Note. *Data were obtained from reference 40–43.

dose of IR, while minimizing the exposure of healthy tissue. Majority of the patients are treated with a dose between 40–60 Gy of radiation, and this dose may be reduced depending on the distance between the radiation source and the target tissue[57]. Like other treatments, radiotherapy also causes serious side effects. Scientists and radiologists take great efforts to direct the beams accurately on tumor cells and minimize the exposure of surrounding normal tissues. Targeted radionuclide therapy is one of the most rapidly developing approach of nuclear medicine. It is based on the use of molecular carriers of radionuclides with high affinity to antigens on the surface of tumor cells. Unlike conventional external beam therapy, targeted radionuclide therapy causes less damage to the normal tissues and allows targeted drug delivery to tumors[1]. It is widely applied in the treatment of the most radiosensitive tumors, particularly leukemias and lymphomas. Unfortunately, even though this new treatment has resulted in longer survival, radiotherapy still causes a growing number of radiation-related secondary cancers.

Occupational exposure of medical staff to radiation is another aspect of this challenge. Although various protection methods have been applied to reduce occupational exposure to radiation, incidence of cancer in medical staff working in an imaging area is high on an average, in comparison to the general population[58].

Taken together, most cancers can be induced by radiation, and a linear dose-response has been noted for most cancers. According to the linear no-threshold model, established by Biologic Effects of Ionizing Radiation VII Committee (BEIR VII), to estimate the relationship between IR exposure and cancer, risk of cancer continues to linearly increase even at low doses of exposure, with no safe threshold; the lowest dose of radiation increases the risk, and there is no safe level of exposure[57].

EFFECTS OF IR ON SOLID TUMORS AND HEMATOLOGICAL MALIGNANCIES

Breast Cancer

Increased risk of breast cancer was reported in women with scoliosis who were repeatedly exposed to x-rays as part of following up of their disorder in their childhood[59]. We have also showed an increased level of 8-OHdG in blood samples a few hours after the whole spine radiography in children with scoliosis[60]. It was reported that breast cancer risk increased significantly in female radiologic technologists who were exposed to daily low dose radiation over several years[61]. Mammographic examination is the best tool for early diagnosis of breast cancer. Although there is no direct observation of breast cancer resulting from routine breast imaging by screen-film- and digital-mammography, the risks and benefits of mammography are the subjects of continuous debate.

Aberrant DNA methylation is proposed as an underlying mechanism in breast carcinogenesis. In a rat model of breast cancer, Loree et al.[62] determined genomic hypomethylation accompanied by the reduction in the levels of DNMT1, DNMT3a, DNMT 3b, and MeCP2. Despite significant differences between different breast cancer subtypes, methylation status of Alu and LINE-1 were found to be changed in invasive breast tumors. It was suggested that LINE-1 hypomethylation is an early event, while Alu hypomethylation is probably a late event during breast cancer progression[63]. It is still unknown whether repeated exposures to x-ray cause the breast cancer through Alu and/or LINE-1

Table 4. The lifetime attributable risk of cancer due to medical radiation exposure in childhood

Data	Study type	References
Pediatric CT scans result in increased lifetime risk for cancer mortality	Retrospective cohort study	46-47
Pediatric CT examinations cause an increased risk of leukemia	Retrospective cohort study	48
Breast cancer risk is increased in subjects with scoliosis	Retrospective cohort study	49-50
Head and neck CT examinations during childhood increases thyroid cancer risk	Prospektif study	51
Pediatric CT examinations cause an increased risk of brain tumors	Retrospective cohort study	48
Ir used for treatment of skin hemangioma during infancy results in intracranial tumors	Retrospective cohort study	52
Ir used for treatment of tinea capitis in childhood results in brain tumors	Retrospective cohort study	53
CT examinations during childhood increases brain tumor risk	Retrospective cohort study	54

hypomethylation or aberrant DNA methylation at the promoter regions of cancer-related genes. In human breast tumors, genes that are involved in cell proliferation, cell cycle checkpoint, and tumor suppression show aberrant DNA methylation in their repetitive elements and promoter regions[64]. However, as far as we know, there is no study examining the effect of x-ray on these methylation changes.

Lung Cancer

During coronary angiography and percutaneous coronary interventions, the lungs are the most exposed organs to radiation. Although lung cancer screening with chest low dose CT was associated with reduced lung cancer and lower overall mortality, full-chest CT scanning, used in diagnosing nodules, and subsequent examinations as follow up, may present an independent risk of lung cancer. It was suggested that current lung cancer screening protocols, if conducted for over 20- to 30-year periods, can independently increase the risk of lung cancer beyond cigarette smoking because of cumulative radiation exposure[65].

Multiple studies showed LINE-1 hypomethylation[16] and a wide range of gene-specific promoter hypermethylation in lung tumors[23]. Changes in the promoter methylation status of tumor suppressors *CDKN2A* and *PTEN*, stimulator of apoptosis *DAP-kinase*, and transcription regulators *SHOX2, SOX17*, and *TCF21* have been reported. Although the contributory roles of ROS damage and aberrant DNA methylation to lung tumorigenesis are well defined, the influence of x-ray on the status of promoter methylation in these genes has not been investigated yet.

Leukemias

Compared to other adult somatic tissues, bone marrow is more sensitive to IR, due to its high turnover rate. Considerable evidence show that children and young adults who undergo multiple CT scans, have an increased risk of leukemia[48]. Aberrant DNA methylation is a contributory factor in the pathogenesis of hematopoietic malignancies. Hypomethylation of satellite repeat sequences was implicated in pericentromeric chromosomal rearrangements found in some human cancers, including leukemias[66]. Important genes that regulate DNA methylation and demethylation are frequently mutated in myeloid malignancies[67]. In addition to global hypomethylation, significant changes in methylation are seen in the promoter regions of various genes involved in cell-cycle control, apoptosis, DNA repair, and tumor suppression (*CDKN2A, BIM, DKK3, O6-MGMT, DBC1/BRINP1*) in leukemia[24].

When DNA methylation status in mouse hemopoiesis in CBA/H (AML-sensitive), and C57BL/6 (AML-resistant) inbred mouse models before and after *in vivo* exposure to a leukemogenic dose of x-rays were examined, bone marrow was found to be one of the most hypomethylated and radiosensitive somatic tissues in adult mammals, and *in vivo* exposure to 3-Gy x-rays induced further hypomethylation of DNA in CBA/H but not C57BL/6 mice[68].

Thymus, which is an important component of hematopoietic tissue, is a primary target for IR-induced carcinogenesis. Both acute and fractionated IR carry a high risk of leukemia and thymic lymphoma. Using an *in vivo* murine model, Pogribny et al.[39] showed that fractionated whole-body exposure to 0.5 Gy x-ray leads to a decrease in histone H4-Lys20 trimethylation, and a significant decrease in global DNA methylation in the thymus; decreased methylation was associated with reduced expression of DNMT1 and, to a lesser extent, DNMT3a. Irradiation significantly reduced in the levels of MeCP2 and MBD2.

Thyroid Cancer

Radiation exposure is a well-known risk factor for thyroid cancer, which is associated with exposure to not only high doses of IR but also to multiple lower doses through medical imaging. Mazonakis et al.[51] reported an increased risk of thyroid cancer from the CT examinations of head and neck during childhood. In a recent meta-analysis, it was documented that exposure of head, neck, and chest to diagnostic radiation, CT scans, and dental x-rays are associated with an increased risk of thyroid cancer[69].

Genetic and epigenetic mechanisms are involved in the development of thyroid tumors. In addition to *RAS*, *RET*, and *BRAF* mutations, epigenetic modifications of certain genes contribute to thyroid carcinogenesis. In papillary thyroid cancer, these driver mutations are frequently associated with aberrant DNA methylation of several genes involved in cellular growth and proliferation. Tumors with mutations in *BRAF, RET/PTC*, and *RAS* showed a 3.6-fold increase in the number of differentially methylated sites, compared with tumors without these mutations[70]. Hypermethylation of the promoter region of tumor suppressor genes *RASSF1A, PTEN*, *TIMP3*, *SLC5A8*, *DAPK*, *RAPβ2*, *CDH1*,

TSH receptor gene *TSHR*, and the DNA repair gene hMLH1, were detected in human thyroid cancers[22,29]. Particularly, changes in the methylation of promoter of *Cadherin 1* (*CDH1*), and a sodium-coupled monocarboxylate transporter *SCL5A8* genes, were associated with the risk of thyroid tumor[22]. Although analyses of various gene-specific methylation in human thyroid tumors are available, the number of genome-wide methylation studies is small; as far as we know, there are only two studies. Rodriguez-Rodero et al.[26] determined changes in genome-wide methylation in papillary, follicular, medullary, and anaplastic thyroid tumors. According to their findings, non-differentiated subtypes are characterized by aberrant hypomethylation rather than hypermethylation of the promoters. They reported four potential oncogenes, *INSL4*, *DPPA2*, *TCL1B*, and *NOTCH4* that are frequently hypomethylated in primary thyroid tumors. Recently, Hesselink et al. found an increasing Alu hypomethylation in distant metastatic differentiated thyroid cancer, poorly differentiated thyroid cancer and anaplastic thyroid carcinoma[71].

Although the effect of low dose IR on thyroid tumorigenesis and the role of aberrant DNA methylation in this process are well documented independently, there is little evidence for the target locations and/or genes that exhibit aberrant DNA methylation due to exposure to low dose of IR. Recently, Penha et al.[72] investigated the effect of IR on gene expression and promoter methylation status of the DNA repair genes involved in homologous recombination (HR) and non-homologous end-joining (NHEJ) pathways in normal differentiated thyroid cell lines (FRTL5 and PCCL3). They found that, acute exposure to x-ray promoted G2/M arrest, but did not alter the expression of genes involved in HR and NHEJ pathways, other than downregulation of *BRCA1* in thyroid cells.

Brain Tumors

Radiation is a major risk factor for primary brain tumors. Subtypes of brain tumor are heterogeneous and differ in etiology. Braganza et al.[73] reported that exposure to radiation was associated with an increased risk for all types of brain- and central nervous system- tumors but radiation carried a greater risk for meningioma compared to glioma; however, the positive association between exposure to IR and risk of glioma was stronger when exposed at younger age, than later in life. Meulepas et al.[54] reported that radiation exposure due to CT scans in childhood increased the risk for both benign and malignant brain tumors.

The genome-wide hypomethylation and gene-specific hypermethylation occur at a high frequency in primary glioblastomas. LINE-1 methylation levels in primary and secondary glioblastomas were lower than in normal brain tissues[74]. *MAGEA1* is one of a group of germline-specific genes that are transcriptionally activated in many types of human cancers. It was shown that *MAGEA1* activation was correlated with genome-wide hypomethylation and increased cellular proliferation. The most severe globally hypomethylated primary glioblastomas are the most proliferative and are associated with demethylation and transcriptional activation of *MAGEA1*[75]. In a recent study, genome-wide hypomethylation detected in brain cancer tissues was found to be almost identical to that found in the blood of the same individuals[76]. Screens for promoter hypermethylation in glioblastomas demonstrated that genes involved in cell-cycle regulation, DNA repair, apoptosis, angiogenesis, invasion and drug resistance (*CDKN2, RB, PTEN, TP53, O6 MGMT*) were hypermethylated[25]. A recent study reported that fractionated exposure to low doses of IR resulted in DNA strand breaks, loss of global genomic methylation and altered expression of methyltransferases and methyl-binding protein MeCP2 in mouse brain[77].

Because of the carcinogenic potential of radiation, the increasing use of medical imaging and therapeutic procedures which involve IR, brings up some questions about the influence of repeated low dose irradiation on increased cancer incidence. A growing body of evidence indicates that cancer risk is increased in people who were frequently exposed to x-rays, especially in their childhood. The role of global DNA hypomethylation in carcinogenesis is well-defined and the phenomenon of x-ray-induced DNA hypomethylation is undeniable[16]. Our current knowledge of the effects of low dose IR on DNA methylation is generally derived from *in vitro* and *in vivo* experimental studies. According to findings, radiation exposure may induce changes in the DNA methylation profile, that may contribute to the development of cancer. Unfortunately, the contributory role of low dose IR on the DNA hypomethylation in various cancers, which is documented in Table 1, has not been investigated yet. Experimental and large-scale prospective cohort studies examining the relationship between DNA hypomethylation and cancer risk are needed in individuals who are frequently exposed to x-rays

for medical reasons. DNA hypomethylation may be a missing link between exposure to medical radiation and cancer development.

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