The Inherited Basis of Human Radiosensitivity

Richard A. Gatti

From the Department of Pathology, UCLA School of Medicine, Los Angeles, CA, USA

Correspondence to: Richard A. Gatti, Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90095-1732, USA. Tel: +1 310 825 7618; E-mail: rgatti@mednet.ucla.edu

Acta Oncologica Vol. 40, No. 6, pp. 702–711, 2001

Certain individuals cannot tolerate 'conventional' doses of radiation therapy. This is known to be true of patients with ataxia-telangiectasia and ligase IV deficiency. Although in vitro testing may not correlate completely with clinical radiosensitivity, fibroblasts and lymphoblasts from patients with both of these disorders have been clearly shown to be radiosensitive. Using a colony survival assay (CSA) to test lymphoblastoid cells after irradiation with 1 Gy, a variety of other genetic disorders have been identified as strong candidates for clinical radiosensitivity, such as Nijmegen breakage syndrome, Mre11 deficiency, and Fanconi's anemia. These data are presented and considered as a starting-point for the inherited basis of human radiosensitivity.

Received 29 November 2000
Accepted 24 January 2001

With the sequencing of the human genome nearing completion, it seems appropriate to consider which genes function to protect us against ionizing radiation (IR) and the types of damage such radiation produces within a cell. However, radiation sensitivity (RS) is not readily measured. It can vary between species, and between cells of different organs within the same species. RS can also differ from one individual to another. That certain individuals cannot tolerate 'conventional' doses of radiation therapy has been the bane of radiation oncologists for many years.

Only within the past few years has it been possible to begin to dissect away the layers of complexity that control RS. In 1967, a seminal observation was made by Gotoff et al. (1) while treating a lymphoma in a patient with ataxia-telangiectasia (A-T); they noted 'an untoward response' to conventional doses of radiation therapy. Subsequently, it was shown that fibroblasts from patients with A-T have an increased RS, using a variety of endpoints such as chromosomal aberrations, colony survival, and radioresistant DNA synthesis. Radiation delivered from various sources and under various conditions was tested, and it became clear that A-T cells had little or no difficulty in repairing the damage created by ultraviolet radiation, unlike cells from patients with xeroderma pigmentosum, but they were exquisitely sensitive to the damage produced by ionizing radiation (2, 3). Although such radiation also produces cytoplasmic lesions, the primary damage caused by ionizing radiation is in the nucleus, in the form of unrepaired double-strand DNA breaks (DSB). Because much of the damage from IR results from the generation of reactive oxygen intermediates, it is not dissimilar from the damage of free radicals generated in the process of metabolizing food (4). Reactive products are increased in A-T cells (5) and the consequent DNA damage they cause generates a ubiquitous substrate for repair enzymes.

DSBs should not be viewed only as pathological lesions in need of 'repair'; physiological DSBs are also created during meiosis (in synapsing chromosomes), and during both V(D)J rearrangement and immunoglobulin class switching (in maturing lymphocytes). It is even possible that DSB rejoining may occur during learning and neurological development, although clear evidence of this has not yet been found.

In yeast, many RS mutants have been defined and these have provided the framework of our understanding of which genes influence cell-signaling pathways to control cell-cycle checkpoints and which genes play a role in the actual rejoining of DSBs. At least five major repair/processing complexes have been described, and these have been operationally divided as those involving *non-homologous end joining* (the Rad50/Mre11/XRS2, DNA-PK/Ku/KARP and XRCC4/Ligase IV complexes) and those involving *homologous recombination* (the Rad51/Rad52/Rad54/Rad55/Rad57 and BRCA1/BRCA2/Rad51 complexes) (6–8). However, these divisions appear to be oversimplified as our understanding increases, and other DNA repair/processing/rejoining complexes are defined. Knockout mouse models for most RS-inducing genes have proved to be embryonic lethals, with only a few exceptions. However, this is not to say that those same essential

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genes cannot cause cancer when altered in a somatic cell lineage, such as with BRCA1 in breast cancer (9).

IN VITRO RADIOSENSITIVITY TESTING (COLONY SURVIVAL ASSAY)

As part of our efforts to ascertain a diagnosis of ataxia-telangiectasia while performing a world-wide linkage analysis/positional cloning project—one that ultimately led to the identification of the ATM gene (10–12)—we established a colony survival assay (CSA) that could be performed on a 5 ml blood sample (13). The cells are transformed with Epstein-Barr virus and then tested in an assay that entails plating two cell concentrations into the two halves of 96-well tissue culture trays (usually 100 and 200 cells per well, respectively). Normal and radiosensitive controls are included in every experiment. Half of the trays are irradiated with 1 Gy, a dose selected empirically from response curves. The trays are incubated for 10 days, after which each well is scored for 'colonies' (i.e. > 32 cells or 5-cell divisions) using MTT as a vital dye. In trays with normal cells that have not been irradiated, roughly 70% of the wells contain colonies after incubation, whereas roughly 35% of the wells contain colonies after radiation; in trays with A-T cells that have not been irradiated, roughly 60% of the wells contain colonies, whereas 5–10% of the wells contain colonies after irradiation. The unirradiated control trays are important for minimizing the effects of slower cell growth (doubling times) in certain disorders, such as A-T, Fanconi's syndrome, and Bloom's syndrome. CSA results range from 38 to 59% for normal cells, and from 7 to 21% for A-T cells (n = 154) (Fig. 1).

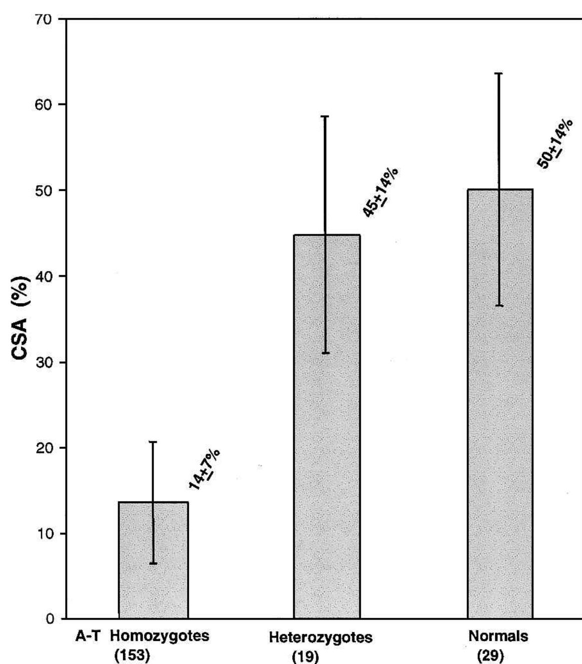

Fig. 1. Mean survival fractions (SF ± SD) after 1 Gy irradiation for A-T homozygotes, A-T heterozygotes and normals.

A-T heterozygotes score as normal under these experimental conditions, although under other colony survival conditions, fibroblast cultures have been shown to be intermediate in radiosensitivity between normal and A-T cells.

Lymphoblastoid cell lines (LCLs) scores in the intermediate range are retested at 0.5, 1, 1.5, and 2 Gy. In all cases observed to date, intermediate results at 1 Gy either remain intermediate at 2 Gy or fall within the RS range of other A-T cells; in no case have we observed an intermediate range A-T LCL scoring in the normal range when retested at 2 Gy (Fig. 2). Thus, the CSA makes a reliable and reproducible starting-point for identifying cells that are unusually radiosensitive in vitro, although it does not necessarily identify in vivo or clinical radiosensitivity. In these experiments, we assume that decreased colony survival is primarily a reflection of increased apoptosis; however, other mechanisms can be envisioned and are being explored in our laboratory.

To expand the clinical interpretation of CSA results, we tested LCLs from patients with disorders that might be

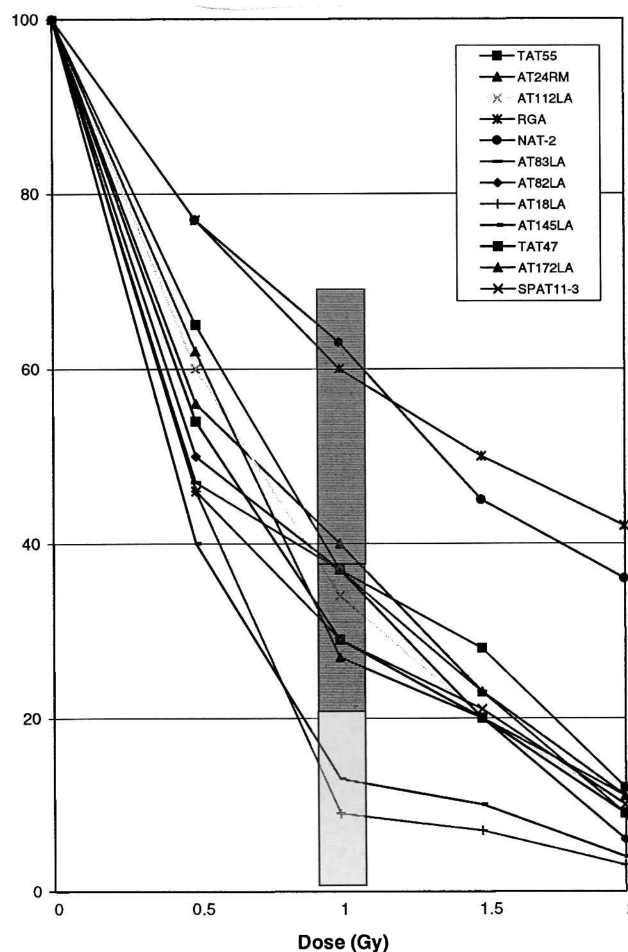

Fig. 2. Dose-response curve of A-T patients with intermediate results.

related to or confused with the phenotype of A-T (Fig. 3). In Friedreich's ataxia, the CSA was normal. Similarly, LCls from patients with Bloom and Rothmund-Thomson syndrome (both RecQ helicase defects) were normal by this assay. LCLs from patients with Fanconi's syndrome (Groups A, B, C, D and G) were as sensitive, or more so, than A-T LCLs. Based on the hypothesis that patients with various primary immunodeficiencies might also be deficient in various aspects of DSB rejoining—and therefore score as radiosensitive by CSA—we tested a variety of these. Five (of 5) patients with X-linked agammaglobulimenima (XLA) were radiosensitive according to the CSA. A SCID LCL with adenosine deaminase deficiency (ADA-SCID) was also radiosensitive. An X-linked SCID LCL was not. LCLs from AIDS patients were included as a control for secondary, non-inherited, immunodeficiency; the scores were normal. This testing is being continued while we attempt to identify a spectrum of genetic disorders associated with RS. However, we can begin to appreciate some of the underlying mechanisms that tie these observations together, by reviewing the molecular pathology for some of the disorders that are associated with RS, as measured primarily by CSA.

ATAXIA-TELANGIECTASIA

A-T cells are the prototype of inherited radiation hypersensitivity (2, 3). Patients with A-T develop a progressive and severe cerebellar ataxia, ocular telangiectasia, immunodeficiency, and one-third develop cancer during their shortened lifetimes (14, 15). These cancers are usually lymphoid. In patients without A-T, ATM mutations or ATM protein deficiency has been reported in association with many malignancies, including breast cancer,

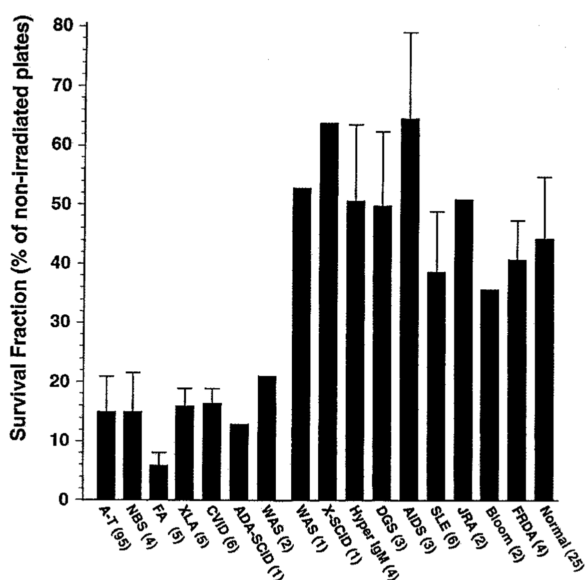

Fig. 3. Colony survival assay (CSA) for lymphoblastoid cell lines (LCLs) from AT and other disorders.

chronic lymphocytic leukemia, mantle cell lymphoma, and T-cell prolymphocytic leukemia. The last of these malignancies is also seen in adolescent A-T patients, usually accompanied by a 14 : 14 inversion that places the TCL1 and TML1 genes from 14q32.1 under the control of the T-cell receptor alpha chain gene (TCRalpha) at 14q11 (16–18).

A-T cells undergo characteristic chromosomal changes. Most commonly observed are translocations involving chromosomes 7 and 14, the breakpoints coinciding with the sites of physiological gene rearrangements at 14q11 (TCRalpha gene complex), 14q32.3 (immunoglobulin heavy chain (IGH) gene complex), 7q32 (the TCRbeta gene complex), and 7p14 (TCRgamma complex) (19–21). Translocations involving chromosomes 2p12 (Ig kappa light chain complex) and 22q11 (Ig lambda light chain complex) have also been reported. These translocations are not seen in A-T fibroblasts, in which an increased frequency of chromosomal aberrations is also observed but is randomly distributed. Telomeric fusions are also observed in A-T cells. Furthermore, accelerated telomere shortening has been described in A-T cells (22, 23).

ATM's closest homologue is yeast TEL-1. When mutated, TEL-1 results in drastically shortened telomeres (from a normal level of $\sim$400 bp, to $\sim$50 bp in TEL-1 mutants) but the TEL-1 mutant is not radiosensitive (24). Thus, telomere maintenance and radiosensitivity must involve different processes—at least in yeast. The substrates of TEL-1 and ATM are also distinctly different. On the other hand, the double TEL-1/MEC1 mutant is radiosensitive. MEC1 mutants show only slight shortening of telomeres ($\sim$350 bp). The human orthologue of MEC1 is ATR (for A-T and Rad3-related) on chromosome 3q22-24 (25), near the recent linkage localization for Seckel sydrome, a disorder with many similarities to NBS (26). ATR appears to play an important complementary role to ATM, during both meiosis (27) and mitotis. In mitosis, whereas ATM reacts quickly to phosphorylate the serine15 residue of p53 in response to ionizing radiation, ATR phosphorylates the same serine site more slowly. ATR also phosphorylates this p53 site in response to single-strand breaks caused by UV radiation. In vivo, ATR phosphorylates CHK1, in response to ionizing radiation (28). ATR $-/-$ knockout mice do not develop beyond embryonic day 7 (29). Telomere length is influenced by many other proteins as well, including EST1, EST-2, EST-3, TCL1, cdc13 ('cap' protein), and KEM3. The 3' overhanging tails generated during telomere synthesis are very recombinogenic and it is not difficult to imagine that even the slightest delay in this process would engender many potential sticky ends for telomeric fusions. The relationship of ATM to telomere integrity is discussed again below, with regard to the Rad50/MRE11/nibrin (R/M/N) complex.

The ATM protein senses or responds to DSB damage and functions as a hierarchical or global PI-3 kinase, phosphorylating p53 and BRCA1 (though several pathways), c-abl, IKK, CtIP, MDM2, CHK2, nibrin, and replication protein A (15, 30, 31). ATM probably autophosphorylates as well. The list of proteins that are phosphorylated by ATM is rapidly expanding; however, distinctions between in vitro and in vivo ATM-dependent phosphorylation are not yet clear for some of these substrates. The consensus phosphorylation site is SQ or TQ (serine-glutamine), with the serine or threonine then being phosphorylated (32). Together, these ATM-dependent pathways provide close control of cell-cycle checkpoints and cell division. Through p53, the signals for apoptosis are also generated. However, it presently appears that in atm −/− knockout mice the degree of apoptosis following radiation varies from one tissue to another, with increased apoptosis noted in the thymus (33) and markedly decreased apoptosis reported in parts of the central nervous system, such as dentate gyrus and cerebellum (34, 35). This begs the question of whether the depletion of Purkinje cells seen in the cerebellums of A-T patients is caused by the decreased apoptosis itself or by allowing a damaged cell population(s) to continue to develop without its appropriate neural interconnections.

Another radiophenotype of A-T cells is radioresistant DNA synthesis (RDS). After irradiation, A-T cells do not postpone DNA synthesis as normal cells do to allow DNA damage to be repaired; they disregard the G1/S checkpoint and enter S-phase unprepared and vulnerable to replicating unrepaired DNA. This checkpoint is controlled primarily by p53, which is phosphorylated in an ATM-dependent manner by at least five molecular interactions. From the shape of the RDS dose-response curve for normal cells, Painter (3) suggested that two mechanisms are most likely involved in RDS at the DNA level: 1) replicon initiation is inhibited at the lower dosages, and 2) chain elongation is inhibited at the higher radiation exposures. In A-T cells, the slope of the high-dose portion of the curve is the same as normal, suggesting that chain elongation is intact in the A-T response. However, at lower radiation dosages, A-T cells are abnormal (the first part of the RDS curve). Painter suggested that, whereas in normal cells a single damaged replicon will inhibit the surrounding replicons from initiating synthesis, in A-T cells this inhibition does not occur, allowing the undamaged replicons to fire anyway. Perhaps a DNA repair complex involving the ATM protein is necessary to coordinate such local events.

NIJMEGEN BREAKAGE SYNDROME

Patients with NBS do not have ataxia or telangiectasia. Instead, they are mentally retarded and have microcephaly (36, 37). They were noted early on to have chromosome 7;14 translocations similar to those of A-T cells. Subsequent testing revealed that NBS cells were also radiosensitive, as measured by colony survival and RDS of fibroblasts, and by CSA of LCLs (Fig. 3). NBS was long considered a ‘variant’ of A-T (A-T Complementation Group V_1) because heterodikaryons produced by Sendai virus or polyethylene glycol fusions of fibroblasts from A-T and NBS patients did not ‘complement’ one another in the RDS assay, implying a common genetic defect or pathway (38). However, further investigations localized the ATM gene to chromosome 11q22.3-23.1 (10), and the NBS1 gene to chromosome 8q21 (39–41).

The failure to complement, as well as the overlap of cytogenetic and RS phenotypes, between NBS and A-T has recently been explained by the fact that phosphorylation of the NBS1 protein, nibrin/p95, is ATM-dependent (42–45). Another syndrome, Berlin breakage syndrome (BBS) (formerly called A-T Complementation Group V_2) includes not only microcephaly and mental retardation, but also anal stenosis and syndactyly (46). Once the NBS1 gene was cloned (47), it was realized that BBS patients also have NBS1 mutations. Almost all NBS and BBS patients of Eastern European ancestry carry the 657del5 mutation in NBS1. Thus, NBS and BBS are really ‘variants’ of nibrin deficiency, not of A-T.

While several molecular pathways and protein complexes may be involved in RS, the R/M/N complex is certainly one of the most important. This complex was first noted by yeast geneticists in Rad50/MRE11/XRS2 radiosensitive mutants (48, 49). When the protein for NBS1 was cloned, a limited but distinct homology with XRS2 was realized (47). The R/M/N complex localizes to the ends of chromosomes and at sites of DBSs, such as during replication and following radiation exposure; it cleaves hairpin structures. Nuclear foci are formed that can be visualized by fluorescent antibodies to any of the components of this complex. Nelms et al. (50) further showed that if chromosomes are damaged by soft x-rays delivered through a grid with open slits, so that the nuclear damage is non-random, the R/M/N complexes form in the regions of the nucleus that were exposed through the slits. Thus, this complex actually migrates to the broken ends of DNA. The R/M portion of the complex is responsible for its endonuclease and exonuclease activity, while nibrin is necessary for localization of the R/M/N complex into the nucleus (48). Ku70 then targets Mre11 to the damaged sites (51). Thus, it would appear that the RS portion of the A-T phenotype occurs primarily because a defective ATM protein kinase fails to activate the R/M/N complex. Apparently, p53 phosphorylation by ATM does not play a major role in RS since p53 −/− knockout mice are not radiosensitive. Since V(D)J rejoining is intact in both A-T and NBS cells (52, 53), the mechanisms

underlying RS must also be distinct from those of gene rearrangement for lymphoid development.

MRE11 DEFICIENCY (AT-LIKE DEFICIENCY SYNDROME)

Mutations in MRE11 were described only recently (54) in two families that were originally thought to have very mild forms of A-T—thus, the name 'AT-like deficiency' syndrome. Fibroblasts and LCLs from these patients are clearly radiosensitive, although perhaps not as strikingly as those of A-T cells. The age of onset of ataxia was similar to that of A-T patients; however, progression of the neurodegeneration was much slower, so that these patients were still working and walking without assistance in adulthood. Although this would be very atypical for A-T patients, our center is following two young men (27 and 30 years old) with a similar phenotype. However, both have two bonafide mutations in the ATM gene and have normal levels of MRE11 on Western blotting of LCL lysates (unpublished data).

In examining the LCL lysates of another 48 patients with atypical phenotypes related to A-T, some of which are also RS by CSA, all had normal MRE11 levels (unpublished data). We also screened the MRE11 gene in these patients for mutations, using both single strand conformational polymorphism (SSCP) and denaturing high-performance liquid chromatography (dHPLC); none were found. Thus, it is unlikely that Mre11 deficiency comprises a significant proportion of the A-T population, certainly less than 1%. The MRE11 gene is located at 11q21, approximately 30 cM proximal to ATM. Unlike NBS patients, MRE11 patients are not from Eastern Europe; the index family is from Pakistan. Unlike A-T patients, the alphafetoprotein levels were normal in all four MRE11-deficient patients, and none had telangiectasias. The similarity of these syndromes should serve to remind physicians and genetic counselors that prenatal testing should not be undertaken in any family with an atypical phenotype unless mutations have been identified in a specific gene, such as ATM or MRE11.

Interestingly, cell lysates from the patients described by Stewart et al. (55) were not only lacking MRE11 protein, but nibrin and Rad50 levels were also markedly reduced on Western blots. These findings were interpreted to signify that MRE11 must play a central role in the stability of the R/M/N complex. Neither MRE11 nor Rad50 levels are altered in lysates from patients with NBS or A-T. In S. cerevisiae, Mre11 functions in DSB repair. Rad50/Mre11/Xrs2 complex yeast mutants have shortened telomeres, slow growth, and are radiosensitive (48, 49, 55–57). It is possible that this complex may function as a kinase that stabilizes telomere integrity. TEL-1 mutants also show markedly decreased levels of XRS2. TEL-1 may open the DNA to insert telomere sequences (or telomerase) and exert its effects on telomere length via the ATM-dependent phosphorylation of the Rad50/MRE11/XRS2 complex, perhaps by first loading the cdc13 'cap' protein.

FANCONI'S ANEMIA

Finding RS in the LCLs of at least five Fanconi (FA) patients (Complementation Groups A, B, C, D, and G) was not surprising, in view of many earlier reports of chromosomal sensitivity of FA cells to ionizing radiation (58, 59). The FA phenotype or syndrome is not easily summarized. Its essence includes patients with a familial pattern of bone marrow failure and cancer susceptibility, accompanied by karyotypes of chromosomal breakage and rearrangements (60). These chromosomal aberrations are further exacerbated by in vitro exposure to DNA crosslinking agents, such as mitomycin C (61, 62). Quadriradial aberrations are characteristic of cells from FA patients, suggesting a cell cycle checkpoint abnormality in G2. At least seven complementation groups are recognized (63). The genes for seven of these have been cloned: FANCC (64), FANCA (65), FANCG (XRCC9) (66), FANCF (67), FANCE (68), FANCD1, and FANCD2.

Presently, the function(s) of the Fanconi proteins remains largely unknown and unrelated to other DNA repair/processing complexes or pathways described to date, primarily because these genes lack sequence homology to other proteins or recognizable motifs. In general, the Fanconi proteins have in common a protective role in handling DNA damage from mutagens like mitomycin C (MCC). Loss of FANCC activity results in a deficiency of radiation- and MMC-induced apoptosis, owing to an inability to activate caspase-3 (69). FANCC protein interacts with FAZF, a zinc finger-containing protein that is homologous with the promyelocytic leukemia zinc finger protein (PLZF) (70). These results suggest that FANCC may be involved in chromatin remodeling via a transcriptional repression pathway. FANCG (XRCC9) also appears to prevent MMC-induced apoptosis. Guillouf et al. (69) suggest that apoptosis induced by MMC and ionizing radiation may be subject to a common regulatory mechanism. It has been further suggested that the FANCA protein may be responsible in some way for transporting other FANC proteins into the nucleus, after it is first phosphorylated (71). However, other evidence suggests that FANCA is primarily a nuclear protein (72). FANCA has two nuclear localization signal motifs as well as a leucine zipper motif. FANCA also binds to SNX5, a member of the sorting nexins (SNX) family, proteins that are thought to play an important role in receptor trafficking between organelles (73). The FANCF gene was recently cloned and found to have no intronic sequences, similar to Ligase IV. FANCF shows homology with ROM, a prokaryotic RNA-binding protein (67). In view of the frequency of myelodysplastic syndrome (MDS) in FA patients (32%), and the poor

prognosis of such patients when MDS develops (5-year survival of 0.09 with MDS vs. 0.92 without MDS), using RS to establish a diagnosis of FA in MDS patients may also be of prognostic value (74). FANCD2 is activated by a nuclear complex of FANCA/FANCC/FANCF/FANCG and colocalizes with BRACA1 in IR-induced nuclear foci and synaptonemal complexes of meiotic chromosomes.

BREAST CANCER

BRCA1 is a tumor suppressor gene responsible for early onset breast and ovarian cancer in susceptible families (75). Approximately 5% of breast cancer patients undergoing radiation therapy have severe reactions to conventional dosage regimens (30, 76–82). Epidemiological studies have also demonstrated that radiation of the breast increases the risk of breast cancer, especially in young women (83). It was thought that because of a consistently observed fourfold increase in breast cancer among heterozygotes from A-T families (15, 30, 84), many breast cancer patients in the general population would be ATM carriers. However, despite a plethora of such studies, the evidence for the latter is still unconvincing. This seeming paradox might be explained if the phenotypes differ for ATM mutations that cause either truncating (leading to degradation of the protein, i.e., null mutants) or missense mutations (with little or no protein degradation, and perhaps a more deleterious "dominant negative" effect). While truncating mutations account for 70% of ATM mutations in A-T patients, they are seldom observed in breast cancer patients from large cohorts (85). Furthermore, breast cancer patients with missense mutations almost never have a family history of A-T. Thus, two types of ATM carrier populations may coexist.

Attempts to identify ATM mutations in groups of breast cancer patients who have experienced untoward reactions to radiation therapy also have been generally unrewarding (86–88). However, the relationship of RS to breast cancer does not necessarily have to involve mutations in the ATM gene itself; the BRCA1 protein is phosphorylated by ATM (89) and is associated with an extremely large DNA repair complex called BASC (BRCA1-associated genome surveillance complex) (90, 91). The complex is ≈ 2 megaDaltons and includes ≈ 40 proteins; those proteins already identified include: ATM, ATR, RAD50, MRE11, nibrin, Bloom's helicase, Replication Factor C, as well as three mismatch repair proteins (MSH2, MSH6, and MLH1). In addition, ATM phosphorylates both CHK2 and CtlP, which bind to BRCA1 in response to radiation; after phosphorylation, BRCA1 dissociates from CtIP and upregulates p21/Waf1 (31, 92). Thus, it is not difficult to imagine that any perturbation of the BASC complex might result in RS, breast cancer, or both. CSA studies of LCLs from breast cancer patients are in progress. BRCA1 and BRCA2 have also been implicated in transcription-coupled repair.

Table 1

Radiosensitivity-associated disorders

Syndrome	Gene	Localization
ADA deficient SCID	ADA	20q13.11
Ataxia-telangiectasia	ATM	11q22.3–23.1
Breast cancer	BRCA2	13q12.3
Breast/ovarian cancer	BRCA1	17q21
Common variable immunodeficiency	???	
DNA-PK deficiency	DNA-PK_{cs}	8q11
Fanconi A	FANCA	16q24.3
Fanconi B	FANCB	?
Fanconi C	FANCC	9q22.3
Fanconi D	FANCD	3p26–p22
Fanconi G (XRCC9)	FANCG	9p13
Ku70	KU70	22q13
Ku86(XRCC5)	KU86	2q33-q35
Leukemia predisposition	Ligase IV	13q33–q34
CHK1 deficiency	CHK1	11q22–q24
Li-Fraumeni syndrome	CHK2	22q12.1
Mre11 deficiency	MRE11	11q21
Multiple endocrine nneoplasia II	MEN2	10q21.1
Nijmegen breakage syndrome	NBS1	8q21
Rad50	RAD50	5q31
X-linked agammaglobulinemia	BTK	Xq21.3
XRCC4	XRCC4	5q13

OTHER DISORDERS ASSOCIATED WITH RS

A full discussion of other genes and syndromes that have been linked to RS in one way or another is not possible within the space limitations of this paper, nor is the list in the Table 1 exhaustive. Some of the other RS-associated genes include: BTK gene (X-linked agammablodulinemia) (13) (see Fig. 3); Multiple Endocrine Neoplasia Type 2 (thyroid carcinoma); ADA (adenosine deaminase-deficient) severe combined immunodeficiency (Fig. 3); CHK2 (= RAD52) (Li-Fraumeni syndrome) (32, 93), Ligase IV, XRCC4, DNA-PK_{cs}, and Ku genes (94–96). RS has also been observed in some patients with common variable immunodeficiency (97, 98). These observations of a concordance of immunodeficiency with RS, and cancer, suggest common underlying mechanisms, perhaps via ATM-dependent phosphorylation of key components of these common pathways or repair complexes. In addition, Meyn (99) identified over 20 cDNA clones (not located at chromosome 11q22–23) that could correct the radiophenotype of A-T cells in vitro. Each of these genes is a candidate for an RS-associated disorder. Other patients who have been described with positive CSA results do not as yet have definable syndromes (100).

CANCER SUSCEPTIBILITY

As can be seen from the above array of RS-associated disorders, many of them also manifest an increased susceptibility to cancer. In some disorders, such as MEN2 deficiency, the cancer penetrance rate approaches 100%. For BRCA1/2, the rate is 50–85%, despite the early embryonic lethality of the BRCA1 −/− mouse (101). In others, such as Fanconi's syndrome and ataxia-telangiectasia, the cancer penetrance is approximately 30%. However, the types of cancer observed are relatively specific for each of these disorders, implying a close link between the genes involved and the mechanisms of oncogenesis. One interesting example: why does a mutation in almost any of the Fanconi complex genes lead almost exclusively to acute myelogenous leukemia or bone marrow failure? This implies that in these patients, who are radiosensitive by CSA and mitomycin C testing, the mitotic process is largely intact while the mechanism by which a stem cell preserves its pluripotentiality depends upon the Fanconi DNA processing complex. This mechanism would appear to straddle the distinction between differentiation and replication. A ligase IV mutation seems to confer a predisposition to leukemia and extreme RS, but no major disruption of immune development and function.

There is also an irony in the fact that the treatment used for many cancers, i.e., radiotherapy, is so closely related to the underlying mechanism that leads to many forms of cancer, i.e. DNA damage. Understanding this relationship is promising for elucidation of new approaches to cancer care. It may also help to identify those disorders in which radiotherapy will further increase the risk of a second cancer (30, 83).

PREDICTING CLINCIAL RADIOSENSITIVITY

To date, there are no reliable tests for predicting clinical RS. Furthermore, RS varies from organ to organ, so that even by testing the skin of a cancer patient for RS, little can be extrapolated from the results that would change the radiotherapy for a particular cancer. CSA testing is a starting-point, in that it identifies individuals with specific genetic disorders that may also manifest clinical radiosensitivity. These disorders can then be genetically linked to regions containing candidate genes for RS, thereby using the Genome Database much like an 'electronic message board'. Genetic screening for the array of disorders discussed herein might also be possible if reliable antibodies were available against each of the cognate proteins for immunoblotting. However, blind-screening with Westerns or even 2D gels is not sensitive enough to allow detection of low-abundance proteins, and some patients may have protein that is non-functional. Expression arrays and mass spectrometry may prove useful, especially for identifying new genes and proteins in RS cells. Thus, by identifying RS in genetically homogeneous patient subsets, we hope to unravel a spectrum of RS-conferring genes.

ACKNOWLEDGEMENTS

The author thanks Patrick Concannon, William McBride, Jon Braun, Ram Iyer, Wayne Grody, Ken Lange, and Helen Chun for critique of this paper, and Xia Sun for assistance with the graphic art. This work was supported by the Ataxia-Telangiectasia Medical Research Foundation, and NIH grants RO1 CA 76513 and RO1 NS35322.

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