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CANCER PATHOGENESIS IN THE HUMAN RecQ HELICASE DEFICIENCY SYNDROMES

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Werner syndrome is an uncommon autosomal recessive disease in which progeroid features are associated with genetic instability and an increased risk of neoplasia. The Werner syndrome locus is one of five human genes encoding a RecQ helicase protein, and Werner syndrome is one of three autosomal recessive syndromes caused by loss of function of a RecQ helicase. This chapter reviews the spectrum of tumors observed in the human RecQ helicase deficiency syndromes, and discusses a model for tumor pathogenesis that draws on our understanding of *in vivo* RecQ helicase function and the consequences of loss of function in specific cell lineages during and following the completion of development.

Introduction

An increased risk of cancer is a consistent and clinically important part of the Werner syndrome (WS) phenotype (reviewed in *11, 19*). The original perception that this increased risk was limited largely to sarcomas has been modified, as additional WS patients and a correspondingly larger number of neoplasms have been documented. The positional cloning and characterization of mutations in the Werner syndrome (*WRN*) and the Bloom syndrome (*BLM*) genes revealed that both of these autosomal recessive genetic instability syndromes were due to loss of function of different human RecQ helicases (*10, 68*). Mutations in one of the three additional members of the human RecQ helicase family, *RecQ4*, were recently identified in a subset of patients with Rothmund-Thomson syndrome (*35*). Rothmund-Thomson syndrome (RTS) resembles Werner syndrome and Bloom syndrome (BS) in having autosomal recessive inheritance in conjunction with genetic instability, an elevated risk of neoplasia and a number of developmental or progeroid features (*48, 66*). The following sections review the spectrum of tumors in the human RecQ helicase deficiency syndromes, and discuss tumor pathogenesis in the context of a model of RecQ helicase function. The reader is referred to chapters by Dr. M. Goto, Dr. Y. Ishikawa and Drs. R.W. Miller and W.W. Nichols for additional information on, respectively, the WS clinical phenotype; cancer in WS patients; and the spectrum of tumors observed in other human genetic instability syndromes.

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Tumor Spectrum: A Clue to Tumor Pathogenesis in the RecQ Helicase Deficiency Syndromes

Useful recent compilations of the histopathologic spectrum — or histopathologic type or subtype distribution — of tumors in WS (*20, 21*), BS (*17*) and RTS (*66*) patients have been updated in Table I. Even a cursory inspection of these data emphasizes the unusual distribution of tumors in patients with different RecQ helicase deficiency syndromes, and the distinctiveness of the spectrum of tumors in each syndrome. A few examples emphasize this point. Sarcomas, the tumors arising from supporting, conducting or blood forming tissues, represent 10–15% of malignant neoplasms in normal adults, but comprise approximately half (range 45–57%) of all tumors in WS, BS and RTS patients. Moreover, the distribution of sarcomas by histopathologic type among WS, BS and RTS patients is considerably different: osteosarcomas and hematologic neoplasms (leukemias and lymphomas) are seen in all three syndromes, but each type of tumor is prominent in only one syndrome (RTS and BS, respectively). Melanoma and meningioma, in contrast, appear to be restricted largely to WS patients. Epithelial neoplasms also occur in patients with RecQ helicase deficiency syndromes, and can comprise up to half (range 43–48%) of all neoplasms within a syndrome. Gastrointestinal and non-melanoma skin neoplasms are seen in all three syndromes, though again each is prominent in only one syndrome (BS and RTS, respectively). Thyroid carcinoma, in contrast, has apparently been observed only in WS patients (*21*).

A second aspect of these tumor spectra, not readily apparent from Table I, is that many of the neoplasms are unusual in terms of site or histology. For example, the melanoma observed in WS patients is acral lentigenous melanoma, an unusual form of melanoma that occurs on nonsun-exposed surfaces such as the palms and soles. A second example, osteosarcoma in RTS, occurs in common long bone sites as well as rare sites such as the patella (*13, 62*). Unusual histologies or subtype distributions are also seen for thyroid carcinoma in WS patients, where follicular thyroid carcinoma histology is prominent, in contrast to the papillary histology observed in most thyroid carcinomas (*29, 45*); and for the distribution of osteosarcoma subtypes in WS patients (Dr. Y. Ishikawa; see chapter this volume). Several embryonal or developmental neoplasms have been reported in patients with RecQ helicase deficiencies. Surprisingly, all of these — retinoblastoma, Wilms tumor and medulloblastoma — have been in BS patients, where they account for 7.3% of tumors (*3, 7, 17, 18*; Table I).

Precursor lesions can provide important clues to tumor pathogenesis at specific sites, and several have been described in patients with the RecQ helicase deficiency syndromes. These include bony abnormalities at sites of osteosarcoma, malignant fibrous histiocytoma or fibrosarcoma in RTS (*65, 66*); extensive poikilodermatous skin changes preceding the development of cutaneous neoplasms, also in RTS (*65, 66*); and myelodysplasia and/or myelofibrosis in BS (*30*), RTS (*56*) and WS patients (*21*). The myelodysplastic syndromes (MDS) are associated with an increased risk of marrow failure and leukemia, and thus may provide an important clue to links between genetic instability and the risk of bone marrow dysfunction or neoplasia (*27, 51*; see below).

Table I effectively highlights the most important questions that we need to

TABLE I. Tumor Spectrum in the Human RecQ Helicase Deficiency Syndromes[a]

Neoplasm[b]	Tumor type and % by syndrome[b]		
	Werner	Bloom	Rothmund-Thomson
Non-epithelial (% of total):	56.6	44.9	56.2
soft tissue sarcomas	15.5	0	5.4
osteosarcoma	6.3	1.8	43.6
melanoma	12.6	0	0
meningioma	11.1	0.9	0
hematologic (total)	11.1	42.2	7.2
leukemia	(6.3)	(19.3)	(1.8)
lymphoma	(<1)	(22)	(3.6)
MDS/MF[c]	(4.3)	(0.9)	(1.8)
Epithelial (% of total):	43.4	47.6	43.6
thyroid	14	0	0
nasal/oropharynx/larynx	2.4	7.3	3.6
gastrointestinal	4.3	18.3	1.8
hepatobiliary	5.3	0	0
breast	3.9	6.4	0
genitourinary	4.8	4.6	0
skin/nonmelanoma	5.8	9.2	36.4
other epithelial	2.9	1.8	1.8
Embryonal (% of total):	0	7.3	0
Wilms tumor	0	4.6	0
retinoblastoma	0	1.8	0
medulloblastoma	0	0.9	0
Tumors examined (*n*):	207	109	55

[a] Tumor spectra were assembled from published summaries (*17, 20, 21, 66*) and additional case studies (see references). Additional reports exist for each syndrome, but were excluded as it was not possible to confirm histopathologic diagnoses or, in some cases, exclude the possibility of double-reporting. A complete listing of case sources for Table I is available from the author.

[b] The histopathologic distribution of tumors and the % of each observed is given by syndrome. Groupings under a specific heading, e.g., soft tissue sarcomas or skin/nonmelanoma epithelial neoplasms, often include several specific histopathologic diagnoses. Total % for each syndrome differs slightly from 100%, due to rounding of % calculations.

[c] MDS/MF, myelodysplastic syndrome/myelofibrosis (see text).

address in patients with the RecQ helicase deficiencies: What accounts for the increased risk of neoplasia? What explains the unusual spectrum of neoplasia including the atypical sites and histologies? and, Why are specific cell lineages or sites affected, while others are apparently spared? Table I also clearly indicates that these syndromes are heritable predispositions to neoplasia (*52*). It is difficult, however, to decide in any formal way how much different the tumor spectrum or risk are as compared with controls (see, e.g., *12*). It should be easier to resolve these issues as genetically defined patient and control populations are developed. The use of genetically defined control or comparison populations should also help resolve questions of genetic heterogeneity: RTS appears to be genetically heterogeneous (*35*), and there are suggestions of genetic heterogeneity in WS as well (discussed in *53*).

A few additional caveats need to be mentioned before attempting to sift the tumor spectra in Table I for clues to pathogenesis. First, the data used to assemble Table I consist largely of case reports that often lack sufficient detail to allow

confirmation and more detailed scrutiny of tumor histopathology. A second problem is that benign neoplasms and precursor lesions of potential pathogenetic importance have not been consistently documented in these syndromes. A final caveat is that the human RecQ helicase deficiency syndromes are comparatively rare diseases, and there are not enough well-documented patients to avoid 'small numbers' problems in which multiple tumors in single patients or the chance occurrence of single neoplasms can have an appreciable impact on tumor spectra.

Functions for the Human RecQ Helicases

Our understanding of *in vivo* functions of the human RecQ helicases and of the importance of genetic instability in human neoplasia provide important clues to neoplasm pathogenesis in the RecQ helicase deficiencies. In addition to the brief summary here, the reader is referred to chapters by Dr. J. Oshima and Dr. H. Ikeda, and to pertinent recent reviews (*8, 9*).

Helicases play a key role in virtually all aspects of cellular nucleic acid metabolism, and often function in conjunction with one or more protein partners (*8, 9, 41*). Three of the human RecQ helicases, RecQL/Q1 (*54, 59*), BLM (*33*) and WRN (*23, 64*), possess 3′→5′ helicase activity, and it is likely that all RecQ helicase family members possess this activity (*34, 36*). WRN is thus far unique in possessing an additional activity, a 3′→5′ exonuclease (*28, 32, 60*). *In vivo* substrate(s) for the human RecQ helicases remain to be defined, but several interesting clues already exist on the basis of *in vitro* assays. For example, purified *Escherichia coli* RecQ protein has the ability to stimulate or disrupt the formation of DNA recombination intermediates, as well as to unwind — and with topoisomerase III catenate — double-stranded DNA molecules (*25, 26*). Several studies have also suggested different template preferences for the WRN and BLM helicase activities (*14, 63*). Associations with other nucleic acid metabolic proteins are also being defined. For example, WRN and BLM may interact with one or more topoisomerases and replication protein A (RPA; *6, 61*).

Clues to *in vivo* function(s) of WRN and of the other human RecQ helicases that would depend on these biochemical activities and associations can be gleaned from the phenotype of bacterial, yeast and human cells that lack RecQ helicase function (reviewed in *8*). Mutants in *E. coli RecQ*, for example, were initially found to be UV sensitive and recombination-deficient in *recBC sbcB*(*C*) mutant backgrounds (*49*). More recently, *recQ* mutants have been found to have increased frequencies of illegitimate recombination (*24*). Similar biochemical, genetic and cellular data exist for RecQ helicase homologues in budding and fission yeast and in *Xenopus* (*8, 67*). These findings indicate that RecQ helicases may play an important role in the replication of specific DNA sequences, and in recombination or recombinational repair events that require at least a limited degree of DNA synthesis (*8, 15*).

Why, given these findings, are there five human RecQ helicases but only three human RecQ helicase deficiency syndromes? A clue to the absence of human RecQL/Q1 and RecQ5 deficiency syndromes can be gleaned from what is known about RecQL/Q1 and RecQ5: both appear to be 'minimal' RecQ helicases that include little flanking protein sequence in addition to a central helicase consensus domain (*34, 54, 59*). Thus human RecQL/Q1 and RecQ5 may be 'housekeeping'

helicases that have few or no *specific* functions. This postulated lack of functional specificity might allow either to be lost with little or no discernable phenotype. However, the double mutant — *recQL/Q1-recQ5* — would likely be embryonal lethal. We should know soon whether *RecQL/Q1, RecQ5* or *QL/Q1-Q5* knockout mice are viable. If this supposition is correct, the clinical and pathologic distinctiveness of the three known human RecQ helicase deficiency syndromes is likely to result from the loss of *specific* functions of WRN, BLM or RecQ4 that cannot be fully compensated for by RecQL/Q1 or by RecQ5.

A Model for Tumor Pathogenesis in the RecQ Helicase Deficiency Syndromes

It is easy to surmise that a genetically determined loss of WRN, BLM or RecQ4 helicase function might lead to the generation of DNA intermediates with the potential to cause mutations or gene rearrangement. A triad of cellular consequences — genetic instability, mutation accumulation and cellular dysfunction, hypofunction or loss — would serve as a strong stimulus for mutation-dependent disease processes such as neoplasia (Fig. 1). The most important determinants of how this 'pathogenetic sequence' affected specific cell lineages are likely to include: the specific role or roles of a particular RecQ helicase in lineage-specific nucleic acid metabolism; additional, lineage-specific roles during and after development; genetic and environmental modifiers on these functions; targets for mutagenesis (e.g., lineage-specific tumor suppressor genes); the potential for somatic selection within a lineage; and time.

Two major scenarios can be sketched that incorporate the essential components and determinants discussed above. Consider first the consequences of loss of function of a RecQ helicase that plays a prominent role in cellular DNA replication. Virtually all cell lineages undergo substantial cell division early in development, and incomplete or aberrant DNA replication due to loss of a RecQ helicase could lead to excessive cell loss and widespread somatic genetic mosaicism in many cell lineages. These problems would persist and worsen in cell lineages that continued to divide

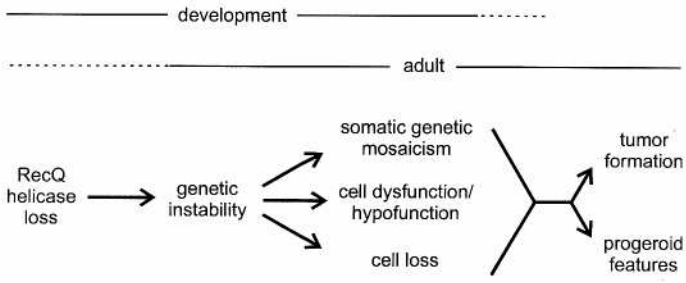

FIG. 1. Model for tumor pathogenesis in the RecQ helicase deficiencies

Loss of RecQ helicase function promotes genetic instability during and following the completion of development, resulting in somatic genetic mosaicism, cell dysfunction or hypofunction and cell loss. The role of a RecQ helicase in specific cell lineages, the extent of mutation accumulation and the balance among altered cell number, cell function and cell turnover or proliferation will determine whether hyperplastic/neoplastic or progeroid features predominate. The importance of time is indicated by the horizontal lines at top.

during adult life, providing opportunities for the somatic selection of neoplastic or pre-neoplastic variants. Consider, for contrast, a second scenario in which there was loss of a RecQ helicase that played a prominent role in recombinational DNA repair. There might be little or no developmental phenotype, as most of the clinical phenotype and disease risk would be determined by a combination of genetic instability, mutation accumulation and opportunities for somatic selection during or following the completion of development. These cellular consequences would, in turn, be influenced by cell division potential of a lineage, by endogenous or exogenous DNA damage levels and by environmental and genetic modifiers. BS and RTS fit most easily within the first of these scenarios, whereas WS fits most easily within the second.

Given these two scenarios, we can ask: Why are BS and RTS patients largely spared CNS developmental sequelae? and: How do WS patients escape early neurodegenerative disease? Functional cross-complementation by another helicase, as described above, may simply be better in the CNS, and the CNS may be further protected by an early cessation of cell replication. A third, more intriguing, explanation of the lack of strong and consistent CNS phenotypes resides in the stringent cell selection that occurs during neurogenesis (reviewed in *50, 55*). The developing CNS may, as a result, be able to tolerate even very high levels of cell dysfunction or loss following the loss of function of a RecQ helicase by consistently selecting for precursors that have retained normal proliferative or functional properties. Thus the CNS could be comparatively 'fault tolerant' by virtue of having found an efficient way to insure normal development by jettisoning or excluding faulty precursors and their progeny. Affected cell lineages in patients with RecQ helicase deficiencies, conversely, may be those in which RecQ helicase function is particularly important; in which RecQ helicase function is strongly modified by environmental exposure or genetic background; or in which there is weak somatic selection during or following the completion of development. Weak somatic selection provides an attractive explanation for the prevalence of sarcomas and non-neoplastic diseases in supporting, conducting and blood-forming tissues in patients with RecQ helicase deficiencies: both somatic genetic mosaicism and ongoing genetic instability could lead to mutation accumulation in these mitotically active or conditionally replicating cell lineages, with an increased risk of cell dysfunction, death or neoplastic proliferation.

Links between Genetic Instability, Mutation Accumulation and Disease Risk

One advantage of the model shown in Fig. 1 is that it makes testable predictions that do not depend immediately upon a more detailed understanding of *in vivo* functions of the human RecQ helicases. We can, as a result, provisionally test ideas about pathogenesis in the context of this model by examining what is known about genetic instability, mutation accumulation, and lineage-specific disease risk in the human RecQ deficiency syndromes.

There are abundant data that document genetic instability in the lymphoid, erythroid and fibroblast lineages in WS (reviewed in *44, 47*) and in BS (reviewed in *16*) patients. Fewer data exist on RTS, but there is evidence for somatic genetic instability in the form of clonal chromosomal rearrangements and somatic mosaicism in the lymphoid (*57*), fibroblast (*43*) and non-keratinizing squamous (buccal) epithe-

lial lineages (*40*), as well as apparent erythroid lineage genetic instability (S.G. Grant, pers. communication; see below). An interesting aspect of the chromosomal instability in RTS is the frequent appearance of chromosome 8 abnormalities including mosaic trisomy 8 and the formation of isochromosomes 8 (reviewed in *40, 57*). It will be important to determine copy number and screen for mutations at both the *RecQ4* and chromosome 8p *WRN* loci in these patients, to determine whether a subset of RTS results from a *RecQ4*/*WRN* copy number imbalance or heterozygosity.

The evidence for *in vivo* genetic instability in RecQ helicase deficiency syndrome patients and the model in Fig. 1 suggest that there may be higher levels of mutation accumulation in cell lineages that show neoplastic or non-neoplastic disease. This has been a difficult issue to address experimentally, as tissue- or lineage-specific measures of mutation production *and* mutation persistence are required. One promising approach to develop these types of data has focused on erythroid lineage genetic instability and disease risk in RecQ helicase deficiency syndrome patients. The erythroid lineage has several advantages for this type of analysis, as it is easily sampled and cell proliferation, differentiation, the kinetics of cell turnover and disease phenotypes are all well-understood (reviewed in *1*). A simple, quantitative somatic mutation assay, the glycophorin A (GPA) variant frequency assay, has been developed that can be used to measure and further characterize *in vivo* genetic instability in this lineage (*31*). We and others have used this assay to document and characterize genetic instability in patients with WS (*37, 44, 46*), BS (*38, 39*) and RTS (S.G. Grant, personal communication).

In a recently completed study we quantified GPA variant erythrocytes in 11 *WS* patients and 11 heterozygous family members that carried 10 different WRN mutations, including three that had been previously shown to confer erythroid lineage genetic instability (*37, 44*). The frequency of GPA Φ/N variant erythrocytes that arise by mutation, loss or epigenetic silencing of a chromosome 4 *GPA* locus was significantly elevated in WS patients and, surprisingly, to a lesser degree in heterozygotes. WS patients alone, however, had a steep positive slope for the increase of these Φ/N variants with age (*46*). These observations indicate that older WS patients have an elevated mutation rate and/or mutation accumulation in the erythroid lineage. The short lifespan of erythrocytes, ~120 days, is particularly advantageous in this type of analysis: mutant red cells must have arisen close to the time of assay, and thus provide a 'snapshot' of mutability during erythroid differentiation that is closely correlated with donor age. The steep age slope for GPA variants in our WS patient cohort is particularly interesting, as it parallels the rapid rise in clinical signs, symptoms and disease risk that begins in WS patients after puberty (*11, 19*).

These and additional GPA data on BS and RTS patients and on patients with the genetic instability syndromes ataxia-telangiectasia (A-T; *5, 22*) and Fanconi anemia (FA; *4, 58*), indicate further how genetic instability may be linked to disease risk in human bone marrow. A-T, BS and FA patients have GPA variant frequencies that are 10-to-100 fold higher than healthy controls, and are at high risk of developing immunodeficiency, leukemia, lymphoma and marrow failure (reviewed in *2, 42*). WS and RTS patients, in contrast, display more modest GPA variant frequencies, and have a correspondingly lower risk of developing bone marrow neoplasia or failure (*21, 46, 56, 66*). The model in Fig. 1 and the steep age slope of Φ/N GPA

variants in WS patients predict that older WS patients should be at higher risk of developing bone marrow dysfunction or neoplasia due to mutation accumulation, and this may indeed be the case: leukemia, myelodysplasia and myelofibrosis represent ~11% of non-epithelial neoplastic or preneoplastic disease in WS patients (Table I). Moreover, as predicted by the tumor pathogenesis model in Fig. 1, WS patients who develop marrow dysfunction or neoplasia do so relatively late in life, with an average age at diagnosis of ~40 years (21 and additional references cited therein).

Prospect

Our working model (Fig. 1) begins to suggest the complexity of tumor pathogenesis in patients with the RecQ helicase deficiency syndromes, and should serve as a guide for future work with patients and on animal models of these fascinating human genetic diseases. Further biochemical and functional analyses of the human RecQ helicases should indicate how the loss of function of different human RecQ helicases promotes genetic instability, and how this loss alters the normal biology of specific cell lineages to increase the risk of developing both neoplastic and non-neoplastic disease.

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