



Mini-review

Tumor development: Haploinsufficiency and local network assembly

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Abstract

According to the current models, tumor development is a continuous process of mutation accumulation, leading to several intermediate phenotypes and final phases of autonomy, unlimited growth and metastasis. One of the most important events in that process is the initial destabilization of cellular pathways that subsequently allow mutations to accumulate. The mechanisms involved in that stage are not clear. In principle, the estimated very low mutation frequency in human or mouse cells would suggest that accumulating the required number of mutations for tumor development should be a statistically unlikely event. However, this theory is contradicted by the high incidence of cancers. Here we discuss the role of protein haploinsufficiency as a contributor to the initial phases of tumor development, and suggest possible mechanisms that might be involved in that process. © 2005 Elsevier Ireland Ltd. All rights reserved.

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1. Introduction

In the past century the advancement of science and technology has significantly extended the human life span and changed the age structure of the population. As a consequence, the incidence of many age-related diseases is increasing sharply, changing significantly the profile of mortality. Cancer is one of the most prominent diseases in humans and currently significant resources are invested in finding an effective treatment for it. Since cancer development is dependant on age, genetic, and environmental factors,

an important unresolved question is to what extent each of these three factors contributes to cancer predisposition and development. The role of environmental factors in some cancer types is well known. Important examples of this in the last 70 years include the sharp decline of death rates from stomach cancers and the sharp rise of lung cancer-related deaths [1]. The link between genetic predisposition and cancer development is also well established. Familial cancers due to an inherited genetic mutation are a small fraction, about 5% of the cancer cases, but represent a very high risk to the individuals involved. However, the role of genetic background in susceptibility to carcinogens is less well established. People differ in their ability to inactivate environmental mutagens

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and to repair the damage caused by them. This leads to the conclusion that there is a segment of the population much more sensitive to carcinogen-induced tumors. Although it is difficult to support this conclusion from statistics related to humans, backcross experiments in mice show that mutagens clearly induce tumors in a specific fraction of the treated animals, confirming the presence of a strong genetic component in response to carcinogens [3].

Despite the apparent genetic and environmental causes, the most significant predictor of cancer is age. Eighty percent of all cancers will arise in individuals aged 55 and older [4]. Age-dependent deterioration in genome maintenance and declining repair capacity is well documented and is most likely a contributing factor [5–7,76,77]. This indicates that cancer at old age may be largely due to naturally occurring age-related genomic instability and that cancer prevention may involve the complex task of reversing age-related changes. In addition, genetic and environmental factors could contribute to the severity of the disease at old age.

2. Tumor related genes

Several mechanisms are currently considered to be involved in cancer initiation and progression [8]. They are all linked to mutations in protein coding genes resulting in deregulation of metabolic or regulatory pathways. However, the known number of human genes in theory is too low to account for the complexity arising in the human cell. Consortium researchers have confirmed the existence of 19,599 protein-coding genes in the human genome and identified another 2188 DNA segments that are predicted to be protein-coding genes [9]. Complex systems principles require the number of regulatory elements to increase as a nonlinear (predominantly quadratic) function of the number of total elements [10,11]. Even if we consider alternative splicing of genes as an option, theoretically the actual number of structural genes in any human cell is too high and the number of regulatory proteins theoretically too low to ensure stable function of any human cell. An alternative regulatory system based on miRNA and siRNA has begun to be described [11] and it is possible that this system could fill the void reserved

for more regulatory elements in cell functions. If that is true it is likely that this system will also be implicated in tumor initiation and progression. This may help explain the uncertainty associated with current cancer models where we frequently refer to ‘genetic background’ as a substantial, but undefined part in the complexity of tumorigenesis.

Currently more than 110 different types of cancer have been described. According to the standard paradigm, each cancer is the result of an accumulation of mutations in tumor related genes, which leads to uncontrolled growth as a consequence of cascade of functional changes. In the process of transformation, cells have to acquire and retain several transitional phenotypes such as unregulated growth potential, external signal blocking and impaired apoptosis among others [2]. The number of genes implicated in tumor development is large. In practice, any cancer type can result from the mutation of particular subset of these genes. However, that set of genes can be different even for the same type of cancer.

We may define two main groups of factors studied in cancer: (1) factors involved in tumor predisposition and initiation and (2) factors involved in tumor progression and maintenance. Studying the factors of the second group has been done routinely. Tumor cells have been isolated and maintained in cultures from which cancer-related genes were identified. This approach, as well as the development of high throughput technologies like gene and protein expression, has expanded significantly our understanding of mechanisms working in developed tumors. An important study [12] summarizing existing tumor-related information indicated that at the time of publication, 291 genes could be designated as cancer genes. Of these 90% display somatic mutations, 20% display germline mutations and 10% show both. Chromosomal translocation creating a chimeric gene is the most common type of mutation class and the most common domain encoded by cancer genes are protein kinases. This information is extremely useful in the development of antitumor drugs and treatments. However, recreating the initial stages of tumor progression using information derived from advanced tumors is very difficult, since the early indicators are masked by the overwhelming mutation frequency of the late phases. These difficulties led to the development of mouse model systems, which are

now used extensively to test the contribution of different genetic backgrounds and mutagens to cancer development [81,82].

3. Role of heterozygosity in tumor development

Mouse models were used successfully to confirm Knudson's 'two hit' theory which requires that both alleles of a tumor suppressor gene need to be inactivated for tumor development. At the same time more data from both mouse models and human tumors pointed out that heterozygosity, leading to protein haploinsufficiency (reduced expression), is also a factor in tumor predisposition and progression. Interestingly, Knudsen's model already linked heterozygosity to cancer initiation and progression. It suggested that heterozygosity for tumor suppressor genes changes the probability of cancer development, but does not functionally contribute to it. However, the new data from human and mouse tumors strongly indicate that heterozygosity leading to haploinsufficiency actually functionally contributes to tumor development. Several excellent reviews [45–49] summarize data from many articles and a complex picture has started to emerge. Haploinsufficiency for *APC*, *Arf*, *ATM*, *BRCA1*, *BRCA2*, *LKB1*, *p53*, *RB* and others has been shown to contribute to tumorigenesis [45]. However, the whole picture of the contribution of heterozygosity to tumor initiation was not apparent until the availability of mouse models where the genetic background was well defined. A summary of some of the available data is shown in Table 1. All data refer to cases where the role of heterozygosity of a gene (one allele inactivated, no protein expression from it) was established as a contributor to tumor initiation. Twenty-four genes implicated in tumor development were found to be heterozygous [13–38]. Twelve of these belong to the group of known cancer genes. The most studied example is *p53*, where heterozygous mice challenged with carcinogens developed tumors. The second group of 12 heterozygous genes implicated in tumor development was not in the cancer gene list and furthermore they were not mutant in the tumors developed as a result of their heterozygosity. Important conclusions from these studies are: *tumor frequency*—heterozygous mice have a higher frequency and earlier onset of tumors

than the wild type mice; *mutagen treatments*—mice haploinsufficient for *TGFb1*, *p18^{Ink4c}*, *p27^{Kip1}*, *p53*, *p21*, *Dmp1*, *SOCS1*, *BubR1*, and *Nbn* develop more tumors than the wild type when challenged with carcinogens (most of the other genotypes were not tested for carcinogen response); *type of tumors*—some tumors developed in heterozygous background are less malignant. The type of tumors (available data for *p53* heterozygous animals only) depends on the strain; *age dependence*—age is an important factor in the type of tumors developed. For example, in young mice heterozygous for *p53* most tumors have both alleles of the gene knocked out, in contrast to old mice that are *p53* heterozygous where only one allele is deleted in most of the tumors [50]. However, the most striking conclusion is that heterozygosity for only one gene is enough to generate conditions for tumor development. If we consider that conclusion applicable to humans, it may dramatically change the potential number of individuals that could be predisposed to tumors. Heterozygous individuals, in contrast to knockout individuals, occur very frequently in the population. For example, the number of *ATM* heterozygous individuals could be as high as 1–2% of US population [83]. Moreover, the same could be true for other genes and consequently the numbers of multiple heterozygous individuals could be substantial fraction of the population. Additionally, the probability of mutation of one allele of a gene as a result of mutagens is much more likely than the knockout of both alleles. Consequently, we may hypothesize that heterozygosity could be a significant contributor to tumor predisposition and development and revealing the mechanisms by which heterozygosity contributes to tumor development is extremely important in cancer prevention and treatment.

4. Mutation frequency and mechanisms for tumor initiation

Since mutation accumulation is the origin of tumors, two very important questions are, what is the mutation rate in human cells and what is the efficiency of DNA repair mechanisms? Estimates based on measurements carried out on the *HPRT* locus of normal human cells indicate that the random mutation frequency is very low. Each cell in a human organism

Table 1

Tumor development linked to protein haploinsufficiency. All data are for proteins the role of which was confirmed in mouse heterozygous models, where tumors formed either spontaneously, either after carcinogen treatment, or both. The genes included in the cancer genes list [12] are marked as 'cancer gene'

Gene	Function	Mouse tumors on haploinsufficient background	References
<i>Annexin 7 (ANX7)</i>	Ca ²⁺ -dependent membrane fusion protein	Spontaneous neoplasms	[13]
<i>APC cancer gene</i>	Nuclear-cytoplasmic shuttling protein, β -catenin chaperone	Intestinal polyps	[14]
<i>BLM cancer gene</i>	RecQ DNA helicase	Lymphoma, <i>challenged with murine leukemia virus</i>	[15]
<i>BubR1</i>	Spindle checkpoint	Lung and intestinal adenocarcinomas, <i>carcinogen induced</i>	[16]
<i>DMP1</i>	Transcription factor	Cancers of many histological types whether untreated or <i>exposed to carcinogens or radiation</i>	[43]
<i>LKB1</i>	Chromatin remodeling, cell cycle arrest, Wnt signaling, cell polarity, and energy metabolism	Hamartomatous polyps, intestinal hamartomas, hepatocellular carcinomas	[17]
<i>Nbn</i> (murine homologue of NBS1) <i>cancer gene</i>	DNA repair	Wide array of tumors affecting the liver, mammary gland, prostate, lung and lymphoma, <i>radiation induced</i>	[18]
<i>NF1 cancer gene</i>	Guanosine triphosphatase (GTPase)-activating protein (GAP) negatively regulates p21ras signaling	Pheochromocytoma, myeloid leukemia	[19]
<i>NF2 cancer gene</i>	Membrane-cytoskeleton linker	Sarcoma	[20]
<i>Nkx3.1</i>	Transcription factor homeobox gene	Prostatic epithelial hyperplasia, prostatic intraepithelial neoplasia	[21]
<i>p18(INK4c)</i>	Negatively regulates cyclin D-dependent CDK4 and CDK6	Pituitary tumors and lymphoma, <i>carcinogen induced</i>	[22]
<i>p27Kip1</i>	Cell cycle regulator	Tumors in multiple tissues, <i>carcinogen or radiation induced</i>	[42]
<i>p53 cancer gene</i>	Transcription factor, apoptosis factor in response to DNA damage and stress	Salivary gland tumors, lymphomas, sarcomas, colon cancer, <i>carcinogen induced</i>	[23,24,50]
<i>PRKARIA cancer gene</i>	Regulatory subunit 1alpha of protein kinase A	Extracardiac tumorigenesis	[25]
<i>PTCH cancer gene</i>	Transmembrane receptor for hedgehog signaling molecule	Medulloblastoma and rhabdomyosarcomas, <i>radiation induced</i>	[26,27]
<i>PTEN Cancer gene</i>	Phosphatase role in apoptosis, cell cycle arrest, and possibly cell migration	Progression of prostate cancer in transgenic adenocarcinoma of mouse prostate model	[28]
<i>RB Cancer gene</i>	Modulating cell cycle progression	Pituitary and thyroid tumors with loss	[29]
Retinoid X receptor alpha (RXRalpha) gene	Regulators of cell growth, differentiation, and apoptosis,	Low grade and high grade prostatic intraepithelial neoplasias	[30]
<i>RUNX1 (AML1, CBFA2) Cancer gene</i>	Heterodimeric core binding transcription factor (CBF) that regulates many genes important in hematopoiesis	Acute myeloid leukemia (AML)	[31]
<i>Sak/Plk4</i>	Polo-like kinases, binds p53	Liver tumors	[32]
<i>SMAD4/DPC4</i>	Mediates the TGFbeta signaling pathway suppressing epithelial cell growth	Gastric polyps and tumors	[36]
<i>Snf5</i>	Core member of the Swi/Snf chromatin-remodeling complex	Malignant rhabdoid tumor	[33]
<i>SOCS1</i>	Suppressor of the cytokine signaling	Hepatocellular carcinomas, <i>carcinogen induced</i>	[34]
<i>TGF-b1</i>	Growth factor	Liver and lung tumors, <i>carcinogen induced</i>	[40]

might accumulate only 1–2 mutant genes during the life span of an individual [54,65]. Additionally, new data suggest that solid and hematopoietic tumors may arise from stem cells [66,67]. The estimated mutation frequency in these cells is two orders of magnitude lower than that of normal cells [68]. At the same time the measured rates of endogenous DNA damage are quite high. It has been estimated that cells produce approximately 10^{10} reactive oxygen species per day, resulting in about 20,000 oxidatively damaged DNA bases per cell per day, including single and double strand DNA breaks, adducts and cross-links [69,70]. Additionally spontaneous reaction of DNA with water causes depurination, in the range of 50,000–200,000 sites per cell [71]. The ROS damage, accompanied by the mutagen effect of external factors like chemicals and radiation, creates a substantial number of DNA damage events. However, the comparison of the induced DNA damage and the actual low mutation frequency in human cells shows that the efficiency of DNA repair mechanisms is extremely high. As a result, cancer should be a rare event. This is at variance with the cancer frequency data. Fifty percent of all Americans will be diagnosed with cancer during their lifetime and 25% will die from cancer. Therefore, the main problem in revealing the mechanisms of tumor initiation is to identify the factors involved in the transition from the low frequency of mutations in normal cells to the high mutation frequency of tumor cells.

The extremely low number of possible mutations in normal cells implies that very small number mutant genes should be involved in tumor initiation. Currently, there are two models that attempt to explain this limitation—the mutator phenotype and aneuploidy [55,56]. According to the mutator phenotype hypothesis, the initial stages of carcinogenesis are the result of mutations in genetic stability genes which increase mutation rates for other genes, and eventually lead to cell transformation and cancer. The aneuploidy model suggests that mutation of a small number of genes required for cell division leads to chromosome breaks or unequal chromosome segregation. This results in genetic instability and the generation of further mutations in multiple genes. Both models propose an explanation of how small numbers of initial mutations could lead to the high subsequent number of mutations needed for cancer

progression. However, these models still require events with very low probability—the complete inactivation of both alleles of a small number of specific genes. Interestingly, there are several other proposals that could be used to address this problem.

The first is that mutations of protein coding genes are not the only mutations that may lead to tumor initiation. Mutations in nonprotein coding genes like miRNA coding genes could contribute to tumor formation. This raises the probability of cancer initiation since the number of genes involved in cancer initiation will increase. Currently, this possibility cannot be proved or disproved.

A second suggestion is supported by the known link between cancer and aging. Cancer is disease of aging and genetic instability is confirmed to be age related. The combination of mutations acquired in the lifetime of an individual with the age-related genetic instability may create conditions for rapid accumulation of new mutations leading to cancer. However, this possibility still does not explain the initial rate of accumulation of mutations in specific genes. It was shown that cancer develops over many years acquiring several transitional phenotypes [1].

A third proposal suggests a significant role for haploinsufficiency in tumor initiation and incorporates two ideas. (a) There is a strong link between deficiencies of functionally related proteins—haploinsufficiency for two or more proteins has a strong additive effect for tumor development. (b) The heterozygosity for many more genes than currently estimated is involved in tumor progression. There is some evidence to support this last suggestion.

5. Additive effect of haploinsufficiency for tumor development

The recent identification of the role of double heterozygosity in tumor development has further advanced our understanding of cancer. Several examples in mouse models have been described and are shown in Table 2 [39–44]. They confirm that double heterozygosity for functionally related proteins have an additive effect on tumor development. For example mice double heterozygous for *Xpc*, and *p53*, genes involved in DNA repair, are more predisposed to UVB radiation-induced skin cancer than *p53* single

Table 2
Role of compound heterozygosity in tumor formation

Gene	Mouse tumors on haploinsufficient background	Mutagen	References
<i>Fen1/Apc</i>	Increased number of adenocarcinomas		[39]
<i>E-cadherin/Apc</i>	Nine-fold and five-fold increase of intestinal and gastric tumor numbers		[40]
<i>Xpc Trp53</i>	Xpc Trp53 double heterozygous mutants are more predisposed to skin cancer than Trp53 single	UVB-radiation	[41]
<i>ATM/Rad9</i>	Increased MEF transformation g-rays	Radiation	[42]
<i>ATM/p53</i>	Increased incidence of mammary carcinomas	Radiation	[43]
<i>NF1/p53</i>	Increased number of sarcomas		[44]

heterozygous mice [41]. Furthermore, the introduction of *ATM* heterozygosity on a *p53* heterozygous background, significantly increased the incidence of mammary carcinoma after X-ray exposure [43]. Heterozygosity for *Fen1*, a DNA replication and repair protein, and *Apc*, a multifunctional protein that plays a role in mitosis, results in an increased number of adenocarcinomas even without exposure to carcinogens [39]. Haploinsufficiency for *ATM* and *Rad9*, another pair of proteins involved in DNA repair, leads to high transformation rates in MEF following X-ray treatment [42]. The overall effect of the double heterozygosity on tumor formation in some of these examples, may be similar to the effect of a complete knockout of a single gene. Importantly, heterozygosity of some of these genes does not contribute to tumor development alone, but if combined with heterozygosity of another gene its contribution is significant as is the case for *Rad9* and *Fen1*. Additionally, several cases exist where heterozygosity for single gene contributes to tumorigenesis on a specific knockout background [45]. All of this evidence further emphasizes the role of heterozygosity in tumor development. It confirms once again that cell functions depend on maintaining the appropriate expression levels of proteins and, more importantly, that cell signaling pathways involve complex interactions between many proteins.

6. Heterozygosity for more genes than currently estimated is involved in tumor development. Role of heterozygosity in tumor related processes

Since tumors result from mutation accumulation, we may broadly define tumor-related processes as those involved with inactivation of mutagens,

mutation repair, DNA replication and cell division. The umbrella classification ‘DNA repair’ is actually a complicated process, involving four different repair systems (NER, BER, Mismatch repair and Recombination repair), checkpoint control and apoptotic pathways. Many proteins control these processes. Currently, 150 human genes can be classified as coding DNA repair proteins [72]. This number includes genes encoding DNA repair enzymes and genes associated with cellular responses to DNA damage, genetic instability or sensitivity to DNA damaging agents. The list of genes involved in tumor-related processes could be expanded significantly, if genes associated with response to external signals, control of DNA replication and cell division are included [51,52]. Heterozygosity for some of these genes has already been shown to contribute to tumor initiation (Tables 1 and 2). Logically, we may suggest that heterozygosity or compound heterozygosity for many others involved in tumor-related processes may have a similar effect. Therefore, a more accurate model for tumor initiation could be one where heterozygosity for single gene or a combination of a small number of heterozygous genes from a large pool of genes, could initiate tumor. This would suggest a strong dependence of cell functions on the expression levels of many proteins and a strong functional link between these proteins. One model for analyzing those links involves the application of biological networks.

7. Analyzing tumor related events. Biological networks and local networks assembly

The complexity of the cellular mechanisms and the appearance of high throughput screening methods in

the recent years required an understanding of the principles of cellular network organization. From work done by several groups [10,53,57–61] it became clear that biological networks are complex, hierarchical and on a local level are usually modular or motif based. One particular mode of organization, the scale-free network, was the predominant type, existing in number of different organisms. Scale-free networks involve different level of connectivity for its nodes—a few highly interconnected nodes communicate with the remaining low interconnected nodes. This type of organization has a significant advantage in the cases where some nodes are inactivated. Even if 80% of randomly selected nodes are inactivated, the remaining 20% will still form a compact cluster with a path connecting any two nodes. The weak link in this type of network is that the inactivation of few highly interconnected nodes will shift the network to small isolated node clusters [10].

It is logical to propose that network characteristics are directly relevant to the status of its elements—in the case of biological networks, the status of proteins representing the nodes. Protein activity, concentration, and spatial distribution will likely play a very important role. In the case of haploinsufficiency we may assume that low concentrations of only one of the highly interconnected proteins could negatively influence network response as a whole.

The principles of general networks require some modifications before applying them to biological networks. Mathematical or computer networks are usually analyzed as already connected structures of elements. Biological networks in many cases are self assembly/disassembly networks. For example, many local networks may be assembled only when they are needed—for instance after DNA double-strand breaks are induced. The requirement for assembly in response to an event at an unknown point in a relatively large (on molecular scale) area, introduces spatial and quantitative limitations on the process. DNA double-strand breaks, for example, are a local event that may appear at any place in the nucleus. A local network has to be assembled at the points of DNA double-strand breaks in order to signal and initiate the repair. The proteins, potential members of the local networks, have to be in close proximity to the break or to be able to translocate quickly to the site. Once assembled, these local networks could

communicate with higher order monitoring networks, which may respond to signals at levels that may have a low threshold. For example, immunofluorescence analysis of cells after radiation-induced DNA double-strand breaks show that many DNA repair proteins, like ATM, p53, MRE11, Rad50 and NBS1, ATR, colocalize and form discrete foci on the sites of DNA damage [73,77,79]. In addition, migration of DNA repair proteins toward the site of DNA damage has been analyzed by FRAP. By measuring the diffusion coefficient of various repair proteins it has been shown that translocation and transient immobilization of Rad51, Rad52, Rad54 as well as the NER repair complex ERCC1–XPF [74,75] occurs on DNA repair sites in mammalian cells.

How expression levels of proteins influence network efficiency in relation to tumor related events can be very well illustrated with the ATM and rad9 expression level dependence of thymocytes apoptosis [42,62–64]. In the example in Fig. 1 different apoptotic rates were measured for ATM wild type, ATM knockout cells and cells single or double haploinsufficient for ATM and rad9 [42]. In wild type thymocytes, ionizing radiation resulted in almost 25% of the cells being apoptotic at the end of the incubation time. However, for ATM knockout thymocytes, only a small fraction of these cells were apoptotic even after a dose of 8 Gy. Interestingly, the ATM haploinsufficient cells showed an intermediate phenotype.

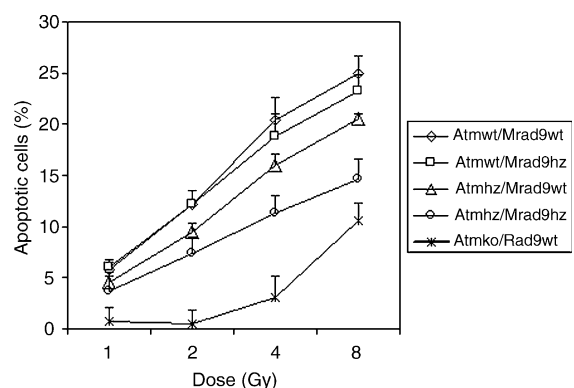


Fig. 1. Apoptosis of thymocytes having different expression levels of ATM and rad9 (from Ref. [42]). Wild type, knockout (for ATM only, rad9 embryonically lethal), single and double haploinsufficient thymocytes were used. Apoptosis was measured by Annexin V-PE and 7AAD staining, 6 h after irradiation with γ -rays (modified from Ref. [42]).

The level of apoptosis in these three different cases reflects differences in the detection of DNA damage. Since ATM is the primary sensor/signaling protein for radiation-induced DNA double-strand breaks, it was to be expected that the damage in wild type cells would be detected and the cells with damaged DNA underwent apoptosis. However, ATM was not present in the ATM knockout cells. Consequently, ATM-dependant DNA repair and apoptotic pathways in these cells were not activated and a significant fraction of the damaged knockout cells remained viable. Most of the cells would likely die by mitotic catastrophe if induced to divide, however, there is a possibility that some of them would survive and could then become transformed at a later stage. The same scenario, although on a much smaller scale, could be applied for the ATM haploinsufficient cells. From the results in Fig. 1 it follows that the same damage that triggered apoptosis in wild type cells resulted in less apoptosis in the haploinsufficient cells. This indicates that small number of these cells survived despite having DNA damage at levels that induced apoptosis in wild type cells.

The apoptosis trigger in these and other cell types is the interaction between several DNA repair proteins. The initial process develops within minutes after DNA damage [84,85] and involves recognition of the break by the Mre11 complex and ATM, and subsequent downstream phosphorylation of Nbs1, Mre11, H2AX, rad9 and other proteins. Once activated, these proteins initiate DNA repair, cell cycle block or apoptosis. Local repair networks comprising these proteins have to be physically assembled at the points of DNA damage. These processes require the presence of the repair proteins in close proximity to the damaged site. If the expression levels and distribution of these or other DNA repair proteins in the nucleus is high enough they will be recruited to these points of damage. In the case of ATM knockout, local networks are likely not assembled or partially assembled, and ATM dependant apoptosis will not be triggered. In the case of haploinsufficiency, the low concentration of ATM (almost 50% of that of the wild type [78] or even less, (down to 10% [80] since cell specific fluctuation in expression levels exist) lowers its distribution density. ATM will not be present in close proximity to all DNA damaged sites and some local networks may not be assembled. The low number of local networks assembled in a small number of haploinsufficient cells will allow some cells to escape

apoptosis and may subsequently result in mutation accumulation. In this case, haploinsufficiency may act as knockout for some local networks because ATM will not be present in damaged sites.

This model would predict that a second haploinsufficiency for a protein involved in the local network could result in further decrease of the number of local networks and further decrease of apoptosis. Interestingly, this is the effect of the rad9 haploinsufficiency—the double haploinsufficient cells show lower apoptotic rates in comparison with the single haploinsufficient cells.

From these data we may suggest that the assembly of the ATM dependant local network is as a critical element in a cells response to DNA damage by radiation. It is possible that the number of local networks assembled on the damage points determines the level of signal generated which in turn controls the induction of apoptosis. If the damage is below a certain level, both wild type and ATM haploinsufficient cells will not respond with triggering of apoptosis. However, in the cases where the level of damage is at, or slightly above the threshold, apoptosis will be triggered in wild type cells only (Fig. 2). At this threshold level some of the damaged points will not be detected in heterozygous cells, and the overall degree of damage may not be interpreted as high enough for an apoptotic response. In contrast, if the damage is high enough, it will trigger response in both wild type and haploinsufficient cells. Therefore, small undetected damage will start to accumulate in the heterozygous cells, induced by concentrations of a mutagen equal to the levels activating apoptotic response in wild type cells.

This model implies different responses to mutagens in wild type and heterozygous cells. Heterozygous cells will tolerate more initial damage and will respond with apoptosis to higher concentrations of mutagens than the wild type cells. Mutation accumulation will be faster and tumors will develop earlier.

In the context of networks, single haploinsufficiencies for a large number of proteins may have an effect on network assembly and stability. According to the scale-free model, the overall effect of a protein on network stability depends on its connectivity—highly interconnected proteins may have the strongest effect on network properties. In this case haploinsufficiency for even one protein may lower the efficiency

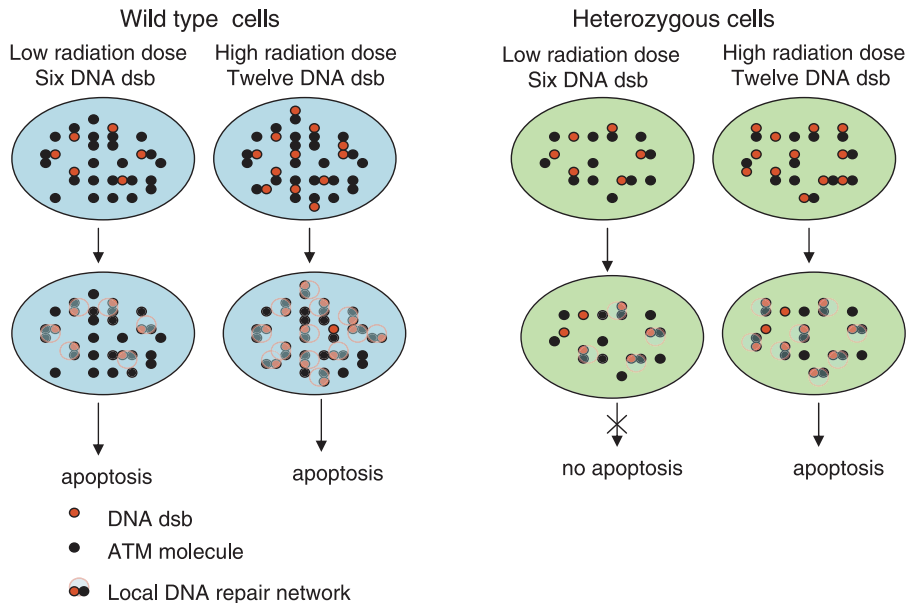


Fig. 2. Local DNA network assembly in ATM wild type and ATM heterozygous cells. The number of ATM molecules is different in wild type and heterozygous cells. Ionizing radiation generates the same number DNA double-strand breaks in both cell types. Hypothetically six or more detected DNA double-strand breaks are required to trigger apoptosis. *Low dose irradiation:* In the *wild type cells* all DNA double-strand breaks are detected by ATM, local DNA repair networks are assembled and since their number is six it triggers apoptosis. In the *ATM heterozygous cells*, as a result of the lower expression levels of ATM, not all of the DNA double-strand breaks are detected, and the number of detected is not enough high to trigger apoptosis. This illustrates how equal damage may trigger different response, depending on the expression levels of ATM. *High radiation dose:* The number of DNA double-strand breaks is high and they cover large areas in the nucleus of both cell types. Despite the low distribution density of ATM in the haploinsufficient cells, six or more breaks are detected. Apoptosis is triggered in both cell types.

DNA repair which, combined with the high rates of DNA damage existing in normal cells, could dramatically change the overall mutation frequency. Interestingly, this suggestion is confirmed by the fact that multifunctional proteins like p53, and ATM, which presumably are highly interconnected, have a very strong effect on tumor formation. Consequently, this theory would predict that tumor initiation should be a more common event, resulting from haploinsufficiency or a combination of haploinsufficiencies of large numbers of proteins, a notion consistent with the statistical rate of cancer in humans.

8. Summary

Mounting evidence suggests that heterozygosity and compound heterozygosity are factors in tumor development. This increases the probability of tumor development. Furthermore, compound heterozygosity

emphasizes the individual predisposition to tumor as a result of the role of a combination of heterozygosity for different genes.

The role of protein haploinsufficiency may be explained from network point of view. The principles of biological networks indicate that specific properties which protect a network's integrity, may in some circumstances contribute to network destabilization. Low expression levels of highly interconnected proteins may change the networks response in the cases when a high number of local networks have to be assembled as a result of a high number of damage incidences. This mechanism may in part account for the higher-than-predicted-rate of cancer in humans.

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