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News and Commentary

Polymorphic variations in apoptotic genes and cancer predisposition

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The concept of cancer-predisposing gene polymorphisms

Normal populational variations of DNA sequence, also called genetic polymorphisms, underlie the intraspecies phenotypic variability. In human, single-nucleotide polymorphisms (SNPs) affect approximately 1 out of 1000 bp; therefore, each subject possesses an unique genetic make-up formed by a combination of a few millions variable nucleotides. No more than 1% of this multitude has a potential to affect directly gene function, but this is sufficient to account for the wide natural genetic diversity of humans in respect to anthropometric, metabolic, behavioral and other features. Although gene polymorphisms do not exert an immediate effect on the well being of their carriers, some SNPs still may play an adverse role by increasing the susceptibility to certain diseases.[1]

Unfavorable SNP combinations are considered to be a major contributor in cancer incidence; therefore, the appropriate studies have gained a high level of attention. Several gene classes have been suspected to influence the tumor risk. In particular, polymorphic xenobiotic metabolizers are believed to be significant modifiers of the susceptibility to the carcinogen-induced malignancies, as they determine actual burden of hazardous substances. For endocrine-related cancers, the polymorphic participants of hormone metabolism have been screened as potential candidates. Despite intensive efforts, only a few gene–disease associations have come out from these investigations, and so the relocation of efforts to other gene classes appears to be a viable strategy. Since recently an increasing interest is paid to the populational diversity in DNA damage response; it is assumed that decreased capacity in elimination of DNA alterations may facilitate the accumulation of somatic mutations and therefore significantly potentiate cancer risk.[2,3]

Deficiency in maintenance of genomic integrity as a possible cause of cancer risk

There are at least two groups of evidence supporting the relationship between DNA damage response variability and cancer risk. First, most of the highly penetrant germline mutations underlying rare hereditary cancer syndromes occur within genes involved in the maintenance of the genomic integrity (BRCA1, BRCA2, MSH2, MLH1, PMS1, PMS2, ATM, p53). It is logical to expect that genes of the same class may also contain more frequent but less penetrant variations associated with tumor susceptibility. Secondly, the impact of populational differences in the extent of DNA damage response is exemplified by phenotyping studies comparing cancer patients *versus* healthy controls.[3]

The cellular response to the DNA damage may involve three distinct processes: cell cycle arrest, DNA repair and cell death. The relationship between these components is not linear, and remains to be studied in greater detail. There are many instances when cell cycle arrest precedes DNA repair and/or cell suicide, thus providing the time to the cellular machinery to choose between these two options, and to prepare for the execution of either of the corresponding biochemical cascades. Sometimes, the programmed cell death is triggered by unsuccessful attempt of DNA repair; in other circumstances, for example, in specific cell types or in case of too heavy DNA damage, cell death constitutes a primary response to the DNA alteration, and its link with DNA repair is less apparent. It is important to acknowledge that the pathways leading to cell cycle arrest, DNA repair or cell death demonstrate extensive overlaps; furthermore, many molecules (p53, BRCA1, BRCA2, ATM, etc.) participate in more than one type of response to DNA damage.[4–7]

DNA damage response and cancer susceptibility: apoptosis *versus* DNA repair

The idea to study the associations between polymorphisms in DNA damage response genes and cancer risk has been met with a great enthusiasm of the research community. For some reasons, the main emphasis has been put on the analysis of SNPs in those genes that participate in the DNA repair processes (OGG1, ERCC1, XRCC1, XRCC2, XRCC3, XPC, XPD, XPF, BRCA2, MRE11, NBS1, Ku70/80, LIG4, RAD genes, etc.).[8–10] Although many promising results have come out, it is becoming evident that the polymorphism of DNA repair genes alone does not provide a satisfactory explanation for the cancer risk variability. Perhaps, accumulation of cancer-associated somatic mutations may occur not only

because of deficiency of DNA repair but also due to reduced ability of the cell death mechanisms to eliminate damaged cells. Although nowadays several forms of programmed and inducible cell deaths, such as apoptosis, necrosis, autophagy, anoikis and mitotic catastrophe, are described, the molecular mechanism(s) of apoptosis is best systematically studied. As mentioned above, DNA repair, genomic instability and apoptosis are intimately linked phenomena, with important implication for the pathophysiology of cancer. However, unlike DNA repair, individual variability in apoptotic response to DNA damage has not been extensively studied in the context of cancer susceptibility. There are several arguments indicating that the research efforts in this direction are likely to be successful.

Phenotyping studies

By now, all major classes of cancer-associated SNP candidates (xenobiotic metabolizers, hormone metabolizers, DNA repair genes) had been selected for intensive research based on the promising results of prior phenotyping studies.[3] Since the phenotyping is capable of measuring an overall functional capacity of a given biochemical cascade, it may provide a valuable overview, whether the studied physiological module indeed possesses a disease-associated significance, and whether the subsequent dissection of this pathway for its polymorphic components is justified.

Only a few case–control studies have compared apoptotic index in cancer patients *versus* healthy controls. Zhao and colleagues[11] analyzed the effect of gamma-radiation on lymphoblastoid cell lines originated from lung cancer patients and healthy subjects; there was a significant decrease of the mean apoptotic index in the former group. Similar results were obtained after measurement of the extent of benzo[*a*]pyrene diol epoxide-induced apoptosis in short-term lymphocyte cultures.[12] Analysis of the response of peripheral blood lymphocytes to gamma-radiation in breast cancer patients revealed the reduction of apoptotic capacity relative to the controls.[13,14] The limitation of these assays is that the tested cells have distinct origin from the target tissue. In this respect, the observation of decreased apoptosis in noninvolved tissue obtained from carcinoma-containing breasts deserves a particular attention.[15] However, while the above-quoted studies on lymphocytes assessed the DNA damage-induced apoptosis, the authors[15] analyzed the spontaneous cell death of normal breast cells.

Thus, the phenotyping tests confirm the association between reduced apoptotic capacity and elevated cancer risk. The question remains whether the individual features of cell death response are attributed to genetic or nongenetic factors. Although the inheritance of apoptotic capacity remains to be proven by direct approaches, such as pedigree or twin analyses, the results of populational phenotyping studies hint at the predominating genetic origin of the observed diversity. Indeed, while the extent of DNA damage-induced cell suicide demonstrates noticeable interindividual differences, intraindividual variability of the apoptotic index is relatively low, even when the repetitive measurements are separated by significant time intervals.[16] Furthermore, an association between allelic variants and the level of apoptosis has already been confirmed for p53 and XPD (ERCC2) gene polymorphisms.[17,18] Therefore, the results of phenotyping studies warrant the systematic analysis of interactions between apoptosis-related SNPs and tumor predisposition.

Studies on rare human diseases

Intriguing observation has been made while studying the apoptotic capacity in rare human diseases, which are characterized by distinct cancer susceptibility. Evident reduction of DNA damage-induced apoptotic response was recorded in patients with Li–Fraumeni syndrome, who carry germline p53 mutation and therefore exert particular proneness to multiple cancer types.[19] Similar observations were made in respect to another cancer-associated disease, Fanconi anemia.[20] Conversely, patients with Huntington disease have both low tumor risk and increased susceptibility to apoptosis.[21,22]

The research of programmed cell death in ataxia telangiectasia (A-T) patients has produced an apparent controversy, as both increased and decreased apoptotic capacity was reported for this disease. It appears that some of the clinical features of A-T may be explained by the elevation of spontaneous cell death. However, when the apoptotic rate is analyzed in the cells exposed to radiation, the A-T homozygotes, and to the less extent heterozygotes, show the deficient response at least in some experimental settings.[13] The latter observation provides a plausible explanation to the association of A-T with the increased cancer incidence.

Polymorphic variations in apoptotic genes in cancer patients *versus* controls

By now, the Arg/Pro codon 72 polymorphism of p53 gene is the only apoptosis-associated SNP, which was subjected to a systematic analysis. The Pro allele of p53 was shown to have a reduced apoptotic capacity as compared to the Arg variant.[17] In accordance with functional evidence, some case–control studies revealed an association of Pro allele with breast, lung and other major cancer types. However, there are many negative reports as well; therefore, the combined analysis of the published data has failed to confirm a tumor-associated significance of the Pro allele.[3] Interestingly, while the disease relevance of the p53 allelism was mainly considered in relation to cancer risk, an exceptionally strong associations have been reported for another apoptosis-related malady, psoriasis: lack of apoptosis-deficient Pro allele was evidently associated with the good therapeutic response to the UV-based therapy (odds ratio $= 22.25$ (95% CI: 7.39–70.31); P-value $= 1.75 \times 10^{-11}$).[23]

The polymorphic substitutions in the XPD (ERCC2) gene have been analyzed primarily in the context of DNA repair studies. However, Asp/Asn polymorphism in the codon 312 may also be relevant to the populational variations in apoptotic capacity, as Asn homozygotes are characterized by increased UV-induced apoptosis.[18] Similarly to the situation with the p53, the relationship between XPD Asp/Asn polymorphism and cancer risk has been demonstrated in

selected case–control studies,[24] whereas the combined analysis of the published data provided more controversial results.[25]

There are some other SNPs in apoptotic genes, which have already been examined in respect to cancer risk; however, their functional significance has not been strictly proven. The most extensive studies have been devoted to the TNFalpha gene polymorphisms, and the associations with several major cancer types have been reported.[26] An involvement in cancer susceptibility has been also suggested for the SNPs located in the FAS promoter region.[12,27,28] There is a series of publications assessing a tumor-associated role of the noncoding G4C14-to-A4T14 allelism.[29] Protective effects have been observed for the DR4 polymorphism and bladder cancer risk, and for the CASP8 variant and breast cancer proneness.[30,31] It is important to emphasize that these initial reports remain to be replicated in independent studies. Furthermore, most of apoptosis-associated SNPs have not yet been subjected to the studies on gene–disease interactions. The number of validated coding SNPs in cell death genes is relatively moderate (Table 1), although this estimate will increase by an order of magnitude if one would also consider noncoding and/or nonvalidated gene polymorphisms (SNP NCBI database, http://www.ncbi.nlm.nih.gov/SNP/).

Future studies

There are several aspects in this field to be studied in greater detail. In particular, the availability of easily accessible, nonexpensive, fairly accurate apoptotic assays makes feasible extended phenotypic comparisons between various categories of cancer patients and healthy subjects. Accurate adjustment of cases and controls appears to be a prerequisite, given the existence of some age- and gender-related variations in the degree of apoptotic response.[16] Studies on lymphomas may have an advantage as compared to other tumor types, because of the shared origin of the target and tested tissues.

Assessment of the functional significance of SNPs residing in apoptotic genes seems to be especially interesting.[17,18] Ideally, data on the functional relevance of particular SNPs may guide the selection of polymorphic candidates for subsequent large-scale case–control studies.[3] However, the available experimental approaches often lack the required precision; therefore, failure to demonstrate the relationship between a given SNP and apoptotic capacity has to be interpreted with caution.

The list of already identified apoptosis-related SNPs (Table 1) is likely to represent the tip of the iceberg, since only a few relevant genes have been systematically screened for genetic variations. With the rapid advances in the methodology of DNA sequence analysis, the prospects for comprehensive SNP portraying of cell death genes are becoming fairly realistic. Probably, a search for new SNPs within this gene class is no less justified than already performed studies on DNA repair polymorphisms.[8–10]

Finally, a low number of case–control comparisons assessing the disease relevance of apoptosis-related SNPs is most likely the main shortage for the time being. In a broad sense, the association studies may represent the most straightforward approach to estimate, whether a given SNP deserves further research efforts; therefore, case–control SNP testing is warranted irrespectively of the availability of the functional data. Since a conclusive epidemiological investigation may require as many as a few thousands tested subjects, the approaches allowing pilot SNP assessment deserve a high attention. For example, it has been suggested that small-scale studies focusing on particularly susceptible categories of tumor patients, such as early-onset and/or familial and/or multiple cancer cases, may quickly provide a valuable preliminary information.[3]

Table 1 Coding nonsynonymous polymorphisms in selected apoptotic genes validated by the analysis of populational frequency[a]

Gene	Polymorphism	Frequency of the variant allele (%)
Bcl2	Thr43Ala	1.3
Bid	Gly10Ser	4.6
Bik	Pro148Leu	5.0
Bcl-x	Gly160Val	4.8
Casp1	Gln37Lys	14.0
Casp2	Leu141Val	4.4
Casp5	Leu13Phe	3.1
	Ala90Thr	42.8
	His152Arg	2.1
	Leu201Val	5.0
	Val318Leu	46.4
Casp6	Glu34Ala	5.0
	Lys35Glu	20.0
Casp7	Glu255Asp	21.1
Casp8	His302Asp	8.2
Casp9	Val28Ala	49.2
	His173Arg	1.1
	Arg221Gln	45.5
Casp10	Ile479Leu	35.2
Fas	Thr16Ala	5.3
	Ile122Thr	1.0
FAIM	Thr117Ala	23.5
	Ser127Leu	14.3
DR3	Gly159Asp	5.3
DR4	Ile33Thr	9.5
	Arg141His	43.2
	Thr209Arg	40.6
	Ala228Glu	12.7
	His297Asn	2.8
	Lys441Arg	3.4
DR5	Leu32Pro	50.0
p53	Arg72Pro	44.5
Survivin	Lys129Glu	9.2
TNFR1	Leu75Pro	3.8
	Gln121Arg	2.4
TRAIL	Glu47Asp	2.1
XIAP	Pro423Gln	35.6

No coding, nonsynonymous, validated polymorphisms reported yet AIF; Apaf1; Bad; Bak; Bax; Bcl-W; Bim; Bok; Boo; Casp3; Casp4; cIAP-1; cIAP-2; DcR1; DcR2; DcR3; DIABLO; FADD; FasL; FLIP; Hrk; Mcl1; Noxa; p73; Puma; TNF; TRADD

[a]SNP NCBI database (http://www.ncbi.nlm.nih.gov/SNP/), status at 1 April 2005; genetic variation was considered as a polymorphism if the frequency of the minor allele exceeded 1%.

Conclusions

While the relationship between populational variability in DNA repair and cancer risk has been extensively studied, the

alternative aspect of the DNA damage response, that is, apoptosis, has been unjustly overlooked in the research of tumor-associated gene polymorphisms. Comparative phenotyping tests, studies on rare hereditary syndromes, as well as some genetic association data indicate that the research on SNPs in apoptotic genes may uncover new low-penetrance determinants of tumor predisposition. Therefore, further analysis of the involvement of apoptosis-related SNPs and cancer susceptibility is highly justified.

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