A Step Toward Genotype-Based Therapeutic Regimens for Breast Cancer in Patients With BRCA2 Mutations?

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The positional cloning of a familial tumor suppressor gene is a monumental task often involving the coordinated efforts of international consortia of many laboratories. The euphoria that accompanies the successful cloning of a cancer-causing gene signals the initiation of many new avenues of study that attempt to define the activity of that gene’s product. These goals are laudatory, because by defining such a function and understanding how it is subverted by mutations, the hope is that eventually this information can be used to develop therapeutic approaches aimed at patients and their family members who participated selflessly in the genetic studies that ultimately led to the identification of the gene.

The biologic functions of gene products are hugely diverse, and our knowledge base of gene function is currently so poor that the experimental pathways that lead to the elucidation of a gene’s function are not necessarily easy to discern. Usually, many years elapsed between the cloning of a familial tumor suppressor gene and the identification of its function. Moreover, although valuable information will be garnered about a gene’s function, translating this knowledge into effective therapeutic regimens, based on the knowledge of an individual’s genetic lesion, is extremely difficult.

However, an article appearing in this issue of the Journal (1), as well as two other articles (2,3), suggests that genotype-based therapeutics for familial breast cancer caused by lesions in the BRCA2 gene may be possible. All of these studies show that cell lines lacking functional BRCA2 are hypersensitive to agents that cause double-strand DNA breaks. Moreover, Abbott et al. (1) demonstrate, in nude mice, that xenografts containing mutant BRCA2 genes are dramatically more sensitive to both ionizing radiation and mitoxantrone, implying that tumors arising in patients with BRCA2 mutations may also be highly sensitive to these therapies. How did this important realization come about so rapidly?

The BRCA2 gene sequence was deposited in GenBank just under 2.5 years ago (4,5). However, the nucleotide sequence provided no clues as to the function of this huge protein, which suggested initially that the experimental pathway to define the function of BRCA2 was likely to be extremely long. As is often the case when such an important disease-associated gene is cloned, many groups (including ours) rushed to clone the mouse homologue (Brca2) and to generate mice lacking this gene. As a prelude to these studies, the mouse gene was cloned (6,7). The sequence of the mouse gene in comparison with its human counterpart provided some surprises. The sequence was poorly conserved for a tumor suppressor gene; for instance, although the genes APC, WT1, NF1, and RB1 exhibit greater than 90% identity between the two species, the BRCA2 gene exhibits only 59% identity with the corresponding mouse gene. This low identity required that the gene be mapped in the mouse genome to provide some confidence that the reported sequences were from the authentic homologue. It is interesting that the poor conservation of the sequence was quite helpful in the analysis of this giant protein, because within the stretches of divergent sequences were several regions that were much more conserved (6,8), suggesting their likely functional significance. BRCA2 was not an exception; we now know that several of these highly conserved domains are functionally important, as shown in Fig. 1.

Among those most relevant to this editorial are the BRC repeats in exon 11 and the highly conserved carboxyl terminus of the protein, both of which have been demonstrated to interact with the recombinational repair protein Rad51 (9,10). It is interesting that, in Paul Hasty’s laboratory, yeast two-hybrid studies (9) that used Rad51 as bait identified the Brca2 polypeptide as a potent interacting partner long before the Brca2 sequence was available in the database. This result and the BRC repeat–Rad51 interaction have been observed by many groups. The Brca2–Rad51 interaction, coupled with the prior observation of the radiation sensitivity of Rad51-deficient mouse embryos (11), made clear suggestions for the appropriate phenotype to examine in Brca2 knockout mice. Acute radiation sensitivity of Brca2-deficient cells was first reported by Sharan et al. (9), by irradiating embryos with a homozygous null Brca2 mutation (Brca2Brdm1). Subsequently, these observations were repeated and confirmed by Connor et al. (12) who used cells with a hypomorphic Brca2 mutation that is compatible with embryonic

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survival (Brca2\textsuperscript{+/−}). Both of these alleles remove the carboxyl-terminal region of the protein, which interacts with Rad51, and the allele described by Connor et al. (12) contains seven of the BRC repeats that interact with Rad51.

Thus, these mouse data led to the suggestion that tumors developing in patients with BRCA2 mutations might also be particularly sensitive to ionizing irradiation or to chemical agents that induce double-strand DNA breaks. However, the poor conservation of the BRCA2 gene poses a potential problem in extrapolating the function of the mouse gene to that of the human gene without an experimental test. Abbott et al. (1) in this issue and Chen et al. (2) have now provided this confirmation by using a pancreatic tumor cell line, Capan-1, that carries the Ashkenazim 6174delT mutation and is missing the other copy. Both groups have shown that BRCA2-deficient Capan-1 cells are highly sensitive to agents that induce double-strand DNA breaks \textit{in vitro}, compared with BRCA2-positive controls, but are no more sensitive than controls when treated with agents with different modes of action (for instance, microtubule or ribonucleotide reductase inhibitors) (1). Moreover, Abbott et al. (1) demonstrate that significant radiation sensitivity was apparent in solid tumors \textit{in vivo} that were generated by injecting Capan-1 cells into nude mice. In fact, necrosis of the tumors was achieved at comparatively low doses of irradiation compared with those required to cause necrosis in BRCA2-positive xenografts.

The mutation in the Capan-1 cells leaves a large truncated Brca2 polypeptide with seven of the BRC repeats intact but without the carboxyl-terminal Rad51-interacting domain. This suggests that the carboxyl-terminal domain is functionally relevant in Rad51-mediated radiation resistance. Chen et al. (2) have suggested that the BRC repeats are functionally important by examining the restoration of radiation resistance in Capan-1 cells containing full-length BRCA2 complementary DNA or versions that have been deleted for the BRC repeats or that contain familial missense mutations. These investigators have speculated that the interaction of the BRC repeats with Rad51 is necessary but not sufficient for radiation resistance. However, these data are negative and inconsistent with the radiation hypersensitivity of the Capan-1 cells in which most of these repeats are intact. Two studies in the mouse suggest that the BRC repeats are not essential for radiation sensitivity. First, the allele described by Connor et al. (12) leaves the BRC repeats intact, presumably in a truncated protein, yet these cells are radiation sensitive. In addition, Paul Hasty’s laboratory (3) has generated an allele missing only the terminal exon of Brca2, which encodes the Rad51-interaction domain that he first defined. Cells missing this last exon are also hypersensitive to agents that create double-strand DNA breaks.

It is likely that both regions are functionally important, because phenocopy of the Rad51 deficiency in mice is attained only when both the BRC repeats and carboxyl-terminal exons have been deleted. Alleles that leave the BRC repeats intact appear to be hypomorphic with respect to Rad51 activity.

Clearly, there is much left to be discovered about BRCA2 function. In particular, it is essential to resolve the basic enigma of why embryonic mouse cells missing Brca2 die, whereas human breast cancer cells lacking BRCA2 can proliferate unchecked.

With respect to the patients, it seems essential now to consider what the practical consequences of radiotherapy are in carriers of BRCA2 mutations. More generally, would approaches that interfere with BRCA2 activity in breast tumors, in combination with conventional chemotherapies, lead to increased cure rates in a disease that will affect so many women?

Even though the therapeutic opportunities for tumors arising in BRCA2 mutation carriers are now apparent, it is also important to consider the possible risks that radiation may pose to BRCA2-deficient cells that may be highly susceptible to the cumulative mutagenic effects of low-dose irradiation, such as that received during mammography screening. Would this type of screen and the recommendation to commence screens earlier and/or more frequently significantly increase the risk of converting these non-neoplastic cells into cancers in these individuals?

References

Lights Flicker on Fluorescence Bronchoscopy in Patients at Risk for Lung Cancer

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The identification of visual, cytologic, and histologic changes that precede the development of cancer has been important for the development of effective treatment and reduction of cervical cancer mortality in the United States (1). The proximal portions of the respiratory epithelium can also be examined visually and sampled using fiberoptic bronchoscopy. The development of techniques to effectively visualize preneoplastic and neoplastic lesions is the first step in applying potential therapies for these lesions. The therapeutic approach may be further screening, local treatment of the respiratory epithelium with photodynamic therapy (2), or systemic treatment with chemoprevention agents (3) in an attempt to prevent the progression of these lesions to invasive cancer.

A new tool to help visually identify preneoplastic and neoplastic lesions in the proximal airways is fluorescence bronchoscopy developed by Lam et al (4,5) at the British Columbia Cancer Agency and marketed by Xillix Technologies as the LIFE™ (Laser-Induced Fluorescence Emission)-Lung Fluorescence Endoscopy System. Kurie et al. (6) conclude in their report in this issue of the Journal that the LIFE bronchoscope did not improve the detection of squamous metaplasia or dysplasia in their population of 39 current or former smokers. This finding differs from other published studies with the LIFE system in populations of smokers and patients with a history of an aerodigestive cancer, suspected lung cancer, or abnormal sputum cytology (4,5,7,8). We propose a number of potential explanations for the discrepancies between this and other studies.

The LIFE system exploits differences in tissue autofluorescence to identify areas of moderate/severe dysplasia and carcinoma in situ in patients with known or suspected lung cancer. It was approved in 1996 for this indication by the U.S. Food and Drug Administration as an adjunct to white light bronchoscopy.

Bronchial epithelial fluorescence is measured in the red (690 ± 10 nm) and green (520 ± 10 nm) wavelengths after stimulation via a bronchoscope by a blue light (442 nm) helium–cadmium laser. A red/green fluorescence ratio is calculated and displayed in real time, with normal tissue appearing green and “abnormal” tissue (moderate/severe dysplasia or carcinoma in situ) appearing reddish brown. Lam et al. (4,5,7,9) reported statistically significant differences in fluorescence ratios between normal tissues and moderate/severe dysplasia and carcinoma in situ. In early clinical studies (4), use of the LIFE system translated into a roughly 50% increase in diagnostic sensitivity for dysplasia/carcinoma in situ with no change in specificity. Use of this device added 8–14 minutes to the bronchoscopy and was not associated with any additional complications.

The other studies (4,7,8,10) with the LIFE system evaluating patients with known or suspected lung cancer identified significantly more moderate/severe dysplasia and carcinoma in situ than white light bronchoscopy. The increases in sensitivity ranged from 38% to 160%, with no change in specificity. A multicenter trial (7) published this year showed that moderate dysplasia or worse (severe dysplasia/carcinoma in situ or invasive carcinoma) was identified in 142 (20%) of 700 assessable endobronchial biopsy specimens and 75 (43%) of 173 patients.

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