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Biomarkers, Regerons, and Pathways to Lethal Cancer

Meng Qiao and Arthur B. Pardee*

Dana-Farber Cancer Institute, 44 Binney St., Boston, Massachusetts 02115

Abstract Cancer is a disease of ''outlaw'' cells that become mutated in regulatory mechanisms. They have lost normal self controls and relationships to the whole organism. Cancers can progress by several pathways from a normal cell to malignant cancer, from bad to worse. Questions about advisability of treatment for some cancers arise from the possibility that they are arrested during progression and so never become lethal. Techniques could be developed to determine the degree of progression and possibility for successful treatment. This article is intended to suggest a way of looking at cancer. It is not a review so references to research articles are infrequent. J. Cell. Biochem. 102: 1076–1086, 2007.

Key words: regerons; cancer; biomarkers

MUTATIONS AND DEFECTIVE REGULATIONS IN THE DEVELOPMENT OF CANCER

Mutations

Cancer is a disease of mutated cells. The origin of a tumor is a single normal cell that has been mutated [Weinberg, 2006]. A new hypothesis is that cancers originate from a small subset of mutated stem cells that can be identified by unique molecular changes (biomarkers) [Wicha et al., 2006]. As a result of numerous mutations the many cells in a tumor develop very different properties. Altered genetics and biochemistry make cancer cells differ from normal cells in structure and functioning so that they grow at wrong times and places. Interactions between nearby cells are seen to change from an organized to an invasive appearance, named the epithelial–mesenchymal transition [Tse and Kalluri, 2007]. Endothelial cells are activated to form new blood vessels (angiogenesis) [Folkman and Kalluri, 2003]. The ability of tumor cells to multiply and spread through the body (metastasize) become increasingly uncontrolled. Metastasis is the major cause of cancer death; it makes surgery ineffective. Other mutations change the targets of therapeutic drugs [Sorlie et al., 2003], and resistance appears against drugs that initially were effective. These differences make it very difficult to kill all the cells in a cancer.

Environmental carcinogenesis suggested that cancers could originate from agents that change a cell's genetic material. Damage to DNA is caused by chemicals or radiation, and is made by duplication of DNA. The errors include substitutions, deletions, duplications, and rearrangements of deoxynucleotides. They arise at many chromosomal positions, and change the functioning of genes located at these positions. In a recent study of breast and colon cancers, an average of 11 genes per tumor were found to be frequently mutated [Benoy et al., 2006].

Multiple mutations are required, accumulation of which takes time. The normal mutation rate is not high enough to produce even one cancer cell in the 100-trillion cells of an individual. However, the mutational process itself can be accelerated by mutation in cancers [Raptis and Bapat, 2006]. This genetic instability can be due to inactivation of DNA repair genes or changes of the telomeres at ends of chromosomes, which prevent proper separation.

Defective Regulation

Positive and negative control systems hold a normal cell in a dynamic steady state. A variety

The article is dedicated to Daniel E. Koshland Jr., outstanding chemist, biochemist, and friend.

*Correspondence to: Arthur B. Pardee, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115.
E-mail: arthur_pardee@dfci.harvard.edu

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of controlling molecules, which are additional to the cell's functional molecules such as enzymes, keep normal cells in balance metabolically and with the whole organism. They could be named regerons, from the latin regere—to lead. They act at many levels—genetic, biochemical, structural, and cellular. A fine example is the control mechanism of cytidine triphosphate synthetase [Long et al., 1970].

Regeron mechanisms are misregulated by mutations that cause metastatic cancer. These thereby allow cells to multiply when and where they should not. Regerons include both tumor suppressor and oncogene proteins, among which retinoblastoma protein (pRb) and p53 protein are the most frequently mutated. Major advances are being made in understanding these mechanisms [Stein and Pardee, 2004]. These changes make metastatic cells lethal. However, understanding their differences from the normal cells provides opportunities for therapeutic intervention.

STEPS TO MALIGNANT CANCER

Cells progress to metastatic cancer through several stages [Foulds, 1954]. Misregulations can increase the number of cells (proliferation), block differentiation into specialized cells, prevent cell death (apoptosis) that can eliminate cancer cells, activate the blood supply required to continue cell growth (angiogenesis), and permit movement out of the tumor (metastasis) [Hanahan and Weinberg, 2000]. The sequence of these several events can vary, and thereby produce different intermediate stages to lethal cancer. As a summary, six pathways of progression from normal cell to malignant cancer are diagrammed [Pardee and Qiao, 2007]. The major three-step pathway is shown in Figure 1.

Step 1—Net Proliferation

The classical hallmark of cancer is the increase of cell number (proliferation). For a cell to make two daughter cells, it must double all of its parts in a sequence of events named the cell cycle and then divide. This process is repeated many times to produce all the cells of an organism. It is similar for normal and cancer cells. Cancers grow because they can initiate their cell cycles independent of external growth factors and escape growth inhibitions by contacting cells [Stein and Pardee, 2004].

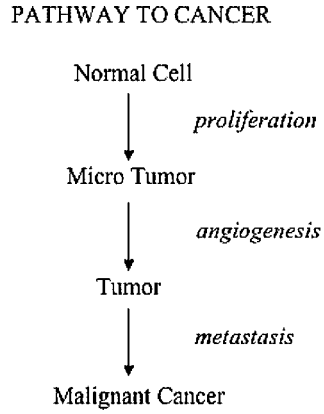

Fig. 1. Pathway to cancer.

Proliferation is usually initiated by binding of external proteins called growth factors to their specific receptors on the cell surface. Cancer cells can become less dependent on these external factors. Epidermal growth factor activates its excessive receptors on many cancers, including breast cancers. The activating information are conveyed into the cell and to the nucleus through signaling cascades. Changes in these multiple steps are often altered during the onset and progression of cancer. Many mutations such as of pRb can bypass the restriction point which normally inhibits cell cycle progression to DNA synthesis. Proliferation probably is the most frequently disregulated initial event. Millions of uncontrolled proliferating cells are then subjected to further mutations.

Apoptosis

A critical balance between cell growth and death is maintained through special molecular mechanisms. Net cell number increases when replication exceeds death. Damage and defects in cell functioning are sensed by molecular mechanisms named Checkpoints, which gives time for repairs to be made by enzymes that are activated. The cell may recover from damage that is not too severe. But greater damage or metabolic stress causes them to commit a "suicide," called programmed cell death (apoptosis) [Lowe et al., 2004]. In cancer, apoptosis is very often decreased and cells that are mutated to resist apoptosis are selected. For example, prostate cells undergo apoptosis when androgen decreases, but prostate cancers can lose sensitivity to androgen and develop resistance to apoptosis. p53, a gene most often altered in cancers, is central to activating apoptosis and

has been called Guardian of the Genome. Other apoptosis related genes, such as BRCA1 and 2 involved in DNA repair, are mutated in some breast cancers.

Senescence

Other processes also oppose proliferation. "Senescence response is a failsafe mechanism that prevents proliferation of cells at risk" [Campisi, 2005]. Normal human cells in culture can stop growing permanently after having passed through about 60 divisions. In vivo, brain cells might last a lifetime, but white blood cells survive for a few hours to few days. Cancer cells, in contrast, are immortalized. Senescence appears in benign but not metastatic cells. Their indefinite proliferation requires activity of telomerase, an enzyme that adds DNA sequences named telomeres to chromosome's ends before cell division. Many kinds of damage and activated oncogenes induce senescence, through the p16/pRb mechanism and p53.

Differentiation

Growth becomes arrested when multi-potential stem cells are modified by epigenetic mechanisms to differentiate and function in specialized ways. Cancer cells that are constantly proliferating lose the ability to differentiate. An early described example is acute promyelocytic leukemia, a disease in which blood cells differentiation from stem cells becomes defective [Lotem and Sachs, 2006]. Tumors contain immature stem-like cells that exhibit differentiation failures [Coletta et al., 2004].

Step 2—Angiogenesis

Mutated proliferating cells become arrested when they form a microtumor, reaching a diameter of about 2 mm. This intermediate stage is not fatal, and can persist for many years. A second step, the production a new blood vessel system (angiogenesis) to supply oxygen and nutrients is essential for further growth to a clinically detectable size [Folkman and Kalluri, 2003]. Before the 1960s, cancer researchers believed that a blood supply reached tumors simply because preexisting blood vessels became dilated. But later experiments showed that angiogenesis was necessary for cancerous tumors to continue growing and spreading. Without angiogenesis, tumors remain dormant or regress.

Step 3—Metastasis

A tumor is a localized excessive growth of cells. It usually is not fatal if detected early and treated immediately. But many of its properties progress from a series of mutations that are followed by selection of those mutated cells that survive and grow faster, and it develops into a mass of differently mutated cells that become difficult to manage. An advanced tumor whose cells have undergone many different mutations can produce malignant cancers when its cells escape from the primary site and move through the blood or lymph system to grow in other places in the body (metastasis). The chance of a tumor developing metastatic activity is high because any of its billion or more cells could be mutated. Metastases interfere with nearby normal body functions, and also can release molecules that modify other cells. The consequences of metastasis are particularly devastating. They are responsible for 80% of cancer deaths. Surgery or radiation becomes far less effective because these are local treatments.

The biology of metastasis is complex and incompletely understood. Several mutations might be necessary for the metastatic switch, a multiple-step process that includes cell's increased migration, motility, escape into the blood, settling into a new site, and then proliferation. Angiogenesis and vasculature are important for the escape of cells [Gupta et al., 2007]. Genes and proteins responsible for these processes are being discovered, such as E-cadherin, beta catenin and GSK-3 kinase [Tse and Kalluri, 2007]. For example, tumor cells are released when cell–cell adhesion molecules with anti-metastatic activities are dissolved by enzymes that increase in cancers. Maspin is a protein that inhibits these proteases; it is eliminated as breast cancers progress. Akt kinase is necessary for metastasis [Ju et al., 2007], and it is activated by phosphorylations in metastatic cells [Qiao et al., 2007]. Metastatic cells are able to proliferate in only those specific organs whose normal cells and conditions permit their attachment and growth into secondary tumors. For example prostate cancer cells frequently metastasize to bone. This is the "seed and soil" hypothesis of metastasis.

Other pathways to metastatic cancer are possible, but are less traveled [Pardee and Qiao, 2007]. Metastatic cancer arising from microtumors may be uncommon because a

microtumor contains far fewer mutable cells than a tumor. An initial mutation to angiogenesis could produce angiomas, small nonmalignant red spots. Alternatively, an initial metastatic mutation could produce an isolated dormant cell [Townson and Chambers, 2006]. Angiosarcomas [Gupta et al., 2007] could be produced when both of these steps are activated, in either order. And their subsequently activated proliferation could produce rare cancers.

EARLIER DETECTION OF CANCERS

Early detection has saved many lives. It is of practical importance because treatment has a greater chance of success if it is applied at an early stage [Suzuki et al., 2006], and it can become ineffective as disease progresses. Cancers discovered by current methods are usually advanced. One notices a symptom or change such as pain, an unusual lump, or bleeding. For example, blood in the feces or changes in bowel behavior raise the suspicion of colon cancer. A skin mole that grows or changes colors may signal melanoma. But even if the patient immediately reports these warning signs to a physician the cancer may already be metastatic and therefore too advanced to be successfully treated.

Breast cancer is tested by frequent physical mammary examinations. Mammography for premenopausal women and ultrasound examinations for postmenopausal women are the most effective methods for breast cancer detection, and indeed mammography is shown by clinical trials to reduce death by 30% [Houssami et al., 2006]. New computerized systems (CAD) are used to examine results of mammography, but it is not yet clear that they improve detection, and false alarms and resultant biopsies are increased. A more reliable, less invasive and cost effective screening method is in need.

Prostate cancer is tested by prostate specific antigen (PSA) in blood [Constantinou and Feneley, 2006]. This protein can increase 5–10 years before clinical symptoms arise, and an average of 17 years before death. But for initial detection this test is not an accurate indicator that cancer is present because PSA is also elevated by an enlarged noncancerous prostate, and so it can give a false positive result [Stenman et al., 2005]. Increases of PSA during a period of "watchful waiting" may indicate that treatment is advisable, because it indicates tumor progression. Elevated PSA can be followed by biopsy sampling of the prostate.

Tumors can be seen as lumps at a billion cells (pea size), and at a thousand billion cells they are lethal. Current methods include physical detection by X-rays, which can reveal advanced tumors, but is not sensitive enough to reveal early cancers. Spiral computed tomography (CT) screening is more sensitive. Another is positron emission tomography (PET). Ovarian cancer screening with high sensitivity is by mass spectroscopy. These current methods are being improved to decrease their error rates and to simplify their application, and to allow tumor localization [Weissleder, 2007].

The PAP smear for cervical cancer and novel inexpensive equivalent techniques have been developed. Other cancers are found less effectively. Colon cancers may be seen by colonoscopic examination and by looking for blood in fecal samples. These can be of high value in practice, but neither test is very sensitive and only three quarters of advanced colon cancer patients were positive for both of them [Lieberman and Weiss, 2001]. The majority of cancers, of both benign and malignant colon cancer patients, were not revealed by colonoscopy, though noninvasive tests showed the K-ras oncogene to be present in eight of nine cases. Lung, colon, ovarian, and pancreatic cancers do not show early symptoms and so are often discovered at an advanced stage when they are very difficult to treat successfully.

Biomarkers

Molecules called biomarkers that differ in amount or structure between cancer and normal tissues are being identified by several molecular biological techniques [Chanin et al., 2004]. They are proposed for detection of early or high risk cancers [Baker et al., 2004], and for guiding early diagnosis and prognostic assessment [Dalton and Friend, 2006]. PSA provides an early major example. There are three main classes of biomarkers—modified DNA sequences, the proteins for which they code, and messenger ribonucleic acids (mRNAs) which are the intermediates between DNA and the proteins [Chatterjee and Zetter, 2005].

Biomarkers include changed sequences of DNA [Sjöblom et al., 2006]. These mutated DNAs are found in genes that cause hereditary cancers, and are valuable for early cancer detection in individuals with a family history

of frequent cancer. Mutated microsatellite DNA is found in cancer cells [Woerner et al., 2006]. The mutated p53 gene is responsible for Li–Fraumeni cancer syndrome. BRCA1 and 2 genes are mutated in 15% of breast cancers. Some African-Americans have a variant DNA sequence that predisposes them to prostate cancers [Amundadottir et al., 2006]. Another example is hereditary retinoblastoma, in which the pRb gene is mutated in one of the two chromosomes, and spontaneous mutation in the second chromosome produces the cancer. Attachment of methyl groups to DNA change gene expressions that can lead to cancer, and were found to provide biomarkers in prostate cancer, and in lung cancer patients' sputum up to three years before clinical diagnosis of high risk individuals who are smokers and/or had been exposed to radon [Palmisano et al., 2000].

Messenger RNA molecules are RNA copies of the 15% or so of all the cell's 20,000 genes that are being expressed. mRNAs provide excellent molecular markers because many differ between normal tissue and cancer. They can be measured very sensitively because their amounts can be greatly amplified by the enzymatic reverse transcriptase-polymerase chain reaction. Identification of mRNA differences depend on techniques for their specific recognition. Microarrays are composed of tens of thousands of short known nucleic acid sequences that are placed at specific locations on a solid surface, to which mRNAs isolated from cells are revealed by specific binding at these locations. Microarrays from normal tissues produce expression patterns that differ from those of major cancers including leukemia, lymphoma, and adenocarcinomas of lung, breast, and prostate [Appasani, 2007]. This technique might change the way cancer is diagnosed, classified and treated in the clinic, although it requires refinements in reliability.

Recently, a new class of RNA molecule, MicroRNAs (miRNAs), has been discovered and used in biomarker studies. miRNAs are small (21–23 nt), single-stranded RNA molecules that specifically regulate the translation of messenger RNAs (mRNAs) in animals and plants [Lee et al., 1993]. It has been shown that miRNAs influence processes such as early development [Reinhart et al., 2000], cell proliferation and cell death [Brennecke et al., 2003]. Mutations that affect expression and activity of miRNAs have been correlated with Chronic lymphocytic leukemia [Calin et al., 2002], Burkitt lymphoma [Metzler et al., 2004], and lung cancer [Johnson et al., 2005]. Furthermore, the expression pattern of miRNAs assayed by microarray were shown to distinguish pancreatic cancer versus normal and chronic pancreatitis, as well as predict patient survival [Waldman and Terzic, 2007]. Although promising, these new findings need to be validated.

Differential display (DD) is a method to identify differentially expressed mRNAs [Liang et al., 2007]. DD integrates two of the most powerful and commonly used molecular biological methods to amplify random sets of mRNAs and then separate the products. The high sensitivity of DD is valuable because little material may be available, sometimes only a dozen cells. A systematic search revealed 13 candidate mRNA markers in blood samples from the vast majority of breast cancer patients versus normal individuals [Martin et al., 2001]. Many changes have been identified. One is found in blood, from a ras oncogene and shown to encode a secreted protein IL-24 which is being investigated as a potential cancer diagnostic marker.

Protein biomarkers can be discovered by a variety of sensitive methods. One method combines immunology with mass spectrometry. Another method for determining multiple proteins in a biological specimen is a microarray based proteomics technology [Posadas et al., 2005]. Proteomic "signatures" of numerous differences in sets of markers can distinguish early vs. late stage disease. Protein microarrays from uterine tissue predict metastasis to lymph nodes. New studies that propose detection of cancer by applying proteomics to serum specimens are promising but need confirmation.

Sample Sources

Bits of surgically removed tissue from cancer or lymph nodes (biopsies) can be used as samples for detecting molecular abnormalities and biomarkers. Disseminated tumor cells were not found by conventional pathology after surgery for early colorectal cancer, but the presence of the mRNA for the structural protein cytokeratin 20 was frequently detected in lymph nodes, and less often in blood or bone marrow. A sampling problem arises from the choice of biopsy location in a nonuniform cancer. There is prognostic value from detection of micrometastatic cells in bone marrow [Braun

et al., 2005]. A signature composed of multiple selected mRNAs from bone marrow of untreated breast cancer patients indicated a 3.5-fold greater risk of death. The risk was 2.9-fold greater as estimated immunochemically by specific binding of an antibody to the target protein. However, problems exist in sampling, such as the choice of biopsy location in a nonuniform cancer.

Body fluids such as blood from patients with a variety of cancers have been found to carry biomarkers. Some are soluble molecules, but they are often located in escaped tumor cells [Anker and Stroun, 2000]. Retinoblastoma mutations, PSA, collagen XXIII and thymosin β15 are being developed clinically as new biomakers [Chatterjee and Zetter, 2005; Hutchinson et al., 2005]. Several markers detected by tissue microarray are predictive of early stage breast cancer in young women [Choi et al., 2005]. New antigens are being found in the blood of patients, of which the most widely applied are CA15-3 and carcinoembryonic antigen (CEA) and they are used to monitor advanced breast cancer therapy; their clinical detection is 2–9 months before recurrence [Duffy, 2006]. Early cervical cancer has been detected immunologically from overexpression of the negative regulatory $p16^{INK4A}$ protein. These immunological tests gave somewhat variable results that in part depend on the antibody concentration used. Alpha fetoprotein is widely applied for detecting liver (hepatocellular) carcinoma caused by hepatitis virus, which is one of the most common cause of death in southeast Asia. Proteins unique to bladder cancer patients are found in blood or urine samples [van Gils et al., 2005]. CA125 is an early ovarian cancer marker. Numerous other markers including oncofetal antigens, glycoprotein antigens, enzymes and isozymes, genes, and cytokines are promising [Zhou et al., 2006].

Mutated mRNA of PSA has been found in prostate cancer cells from the blood of prostate patients. Peripheral blood of colon cancer patients contains tumor-related mRNAs, which if adequately identified could provide an analysis to replace colonoscopy. The PTEN and BRCA1 genes and chromosome region 7q22-23 are commonly altered in higher grade prostate cancer patients' blood, correlated with early death. After they have mutated to lose PTEN, primary tumor cells frequently escape into blood [Schmidt et al., 2006]. Circulating tumor cells have been found with DD [Fournier et al., 1999]. In a recently published paper, the molecular signature to predict good prognosis of breast cancer patients was uncovered using 3D culture models of differentiating nonmalignant human mammary epithelial cells. In this study, genes that correlated with growth arrest of nonmalignant cells and therefore might be good targets to treat early disease were identified [Fournier et al., 2006].

mRNAs unique to prostate cancer patients have been identified in urine samples [Bai et al., 2007]. Biomarkers for early detection of lung cancer are being sought in serum, sputum, and exhaled breath [Chanin et al., 2004]. Ki-ras mutations were found in fecal samples from colorectal cancer patients [Smith-Ravin et al., 1995]. Advantages of such noninvasive sampling include safety, convenience, low sampling bias, and reduced cost.

A problem is to find the rare biomarkers that are common to a kind of cancer. A single marker is not often useful, an exception being PSA. Far more random changes arise from the multitude of mutations of random genes than from the genes that contribute to advanced cancers [Sjöblom et al., 2006]. Sets of commonly altered markers, cancer signatures, are therefore needed for detection. Strong signals for the few markers that are present in most of the cancers are retained by pooling multiple samples and analyzing a small fraction, whereas infrequent markers are diluted [Bai et al., 2007]. A rapid method has been developed for high throughput screening for mutations in oncogenes of many cancers [Thomas et al., 2007]. A signature set of selected mRNAs in small blood samples taken from breast patients was identified by DD. This entire set of markers was then examined by an array technique [Martin et al., 2001]. Signatures from frozen specimens of early lymph node negative cancers were composed of 76 mRNAs [Foekens et al., 2006].

BIOMARKERS AND THERAPEUTIC DECISIONS

Earliest possible detection should improve the chance of successful therapy. But the ability to detect early very small tumors creates a problem. Biomarkers also can detect small cancers including microtumors and micrometastases that frequently do not progress and have a low risk of subsequent lethality [Folkman and Kalluri, 2003]. This leads one to inquire how to

distinguish the dangerous cells from microtumors that may not need to be treated.

Lung cancer unfortunately remains a very lethal disease. Current screening methods are unable to detect tumors when they are localized and treatable by surgery. While only 10% of lung cancer patients survive, it was found by CT scans in 1/60 randomly selected individuals, 80% of whom were immediately treated survived for 10 years. But this value of early detection has been questioned. Another recent study reported that lives were not saved by detecting lung cancers with CT scans; the same fraction of people who had CT scans died as in a control group that did not have scans [Bach et al., 2007]. An explanation for these different conclusions is that CT scans reveal many nonlethal small cancers that are included in the earlier calculation.

The value of early detection also depends upon whether something can then be done to treat the cancer. Should therapy always be applied after cancer is detected? This decision is important because risks from surgery or chemotherapy could outweigh benefits, for example, prostate cancer in elderly men is not always treated immediately, but only after a period of "watchful waiting." Surgery is the primary method for treating cancer. The standard for patients with early breast cancer is surgical removal with a wide local margin followed by X-radiation [D'Souza and Baum, 2006]. But surgery can be effective only if the cancer has not already metastasized. Surgical procedures therefore are often followed by radiation, which sometimes can produce secondary cancers.

Chemotherapy can be used, as when surgery and radiation are not effective. Treatment with drugs or with antibodies poses many difficult problems. Drugs that kill tumors can cause mutations which transform normal cells to cancer, and develop resistance to any one drug. These survivors can develop into treatment-resistant disease. Quality of life after completion of treatment is an important consideration. Side effects from chemotherapy can cause major problems for cancer patients. Some anti-cancer drugs are DNA building block analogs (5-fluorodeoxyuridine, cytarabine) and inhibitors of DNA synthesis (methotrexate) that kill normal blood-forming and intestinal cells whose DNA is frequently duplicated. And many anti-cancer drugs are marginally effective, extending life for only a few months. We need sensitive biomarkers that determine whether or not various treatments are or are not likely to be of benefit.

PREDICTIVE BIOMARKERS

We also need biomarkers to distinguish lethal cancers that will progress from the less dangerous static microtumors. There are already many examples of changes that correlate with a cancer's lethality. Breast cancer patients were classified into poor and good prognosis groups by microarray analysis of their nonmalignant epithelial cells [Fournier et al., 2006]. Mutations in the p53 gene, central to killing of cancer cells, indicate shorter survival and resistance to chemotherapy. PTEN is frequently absent in advanced cancers and indicates poor prognosis. Cyclin E, which is critical for controlling cell growth changes in both amount and structures as breast cancers progress, and this change correlates with development of resistance to chemotherapy [Hunt and Keyomarsi, 2005]. Patients with long versus short times to recurrence and survival have been identified with microarrays of sets of mRNAs from breast tumors. The progesterone receptor and protein Ki-67, as well as tumor stage, and lymph node status are markers detected by immunological microarray predictive of distant failure of early stage breast cancer in young women [Choi et al., 2005]. Anti-apoptotic biomarkers include increased transcription factor NF-κB, involved in resistance of cancer cells to apoptosis [Biswas et al., 2004]. Changed proteins that are involved in cell proliferation indicated metastases of prostate cancers.

A hypothesis is that treatable cancers do not yet have the mutations that bring them close to malignancy. A comparison of biomarkers related to the three steps of Figure 1 might together determine whether a small tumor is likely to be dangerous. Can biomarkers be found for each step? Several lines of evidence suggest that biomarkers for angiogenesis [Naumov et al., 2006] and metastasis, such as maspin [Bailey et al., 2006] and phosphorylated Akt [Ju et al., 2007; Qiao et al., 2007] may be useful in determining the aggressiveness of a cancer. A recent article makes similar suggestions [Mol et al., 2007].

FINALE—SOME OTHER USES OF BIOMARKERS

Biomarkers can be applied to other problems. Cancers of individual patients, even those

arising from the same organ, are not created by the same molecular events. They respond differently to therapies. This choice is complex for invasive breast cancers which have different clinical properties. In 70% of breast cancers, receptor protein for estrogen (ER+ tumors) is elevated; their growth is thereby excessively stimulated. Tamoxifen is an antagonist of estrogen that acts against only these ER+ tumors. Most other breast cancers are stimulated by growth factor proteins present in blood, and these express a different set of markers. Patients whose cancer has many copies of tHER2 benefit from treatment with an antibody named Herceptin. Many prostate cancers require androgen for growth and can be treated with drugs that decrease or inhibit androgen, but others are hormone independent and they require chemotherapies.

The goal of individualizing therapy is to provide each patient with a specific most effective treatment, rather than a one-fits-all general therapy. Prediction of what treatment could be effective against an individual patient's cancer are being developed from biomarkers that identify oncogenic pathways, subtypes, and responding tumors [Bild et al., 2006]. A set of biomarkers in addition to the currently used criteria of cancer stage and grade may be helpful, especially for intermediate grade (grade 2) cancers [Sotiriou et al., 2006]. Node-negative patients were divided into positive vs. negative groups as determined by the expression of 70 genes in their removed tumors. Twenty-three percent with a positive pattern had recurrence within 5 years versus only 5% of the negatives.

Detection of any cancer-related gene or gene product such as a messenger RNA or protein (such as PSA) in a patient's blood would indicate progression of disease and recurrence [Baker et al., 2004]. Arbitrary correlations of markers with clinical outcomes will be needed. Early detection is a good start, but usefulness of any method depends on whether subsequent treatments are effective.

FUTURE OF BIOMARKERS

Routine cancer detection tests are made possible by examining sets of established biomarkers in a small sample of a patient's blood during periodic checkup tests. An estimated 1,000 commercial genetic tests are being developed for determining an individual's gene composition and for detecting medical problems. A first genetic test to predict risk of relapse of breast cancer (MammaPrint) is approved by the FDA. Mammaprint uses the 70 genes published [van de Vijver et al., 2002]. Another commercial prognostic test for ER positive breast cancer is OncotypeDX.

This is just a start. Realizing the potential of biomarker applications is a challenge. Many practical problems will have to be addressed before selective and sensitive genomic tests can be applied clinically. How early a cancer can be found requires more research. Sensitivity of the tests need to be improved. A single change, of which many are seen in some but not in other cancers, is not adequate. A compelling panel of frequently altered biomarkers need to be identified. These differences will need to be significant, to reveal the few cancer cells in many normal ones. Techniques must be standardized and optimized. Application of markers will require a database connecting their profile and the results of clinical treatment. Although some of these are promising their reliability has not been determined, nor have they been legally regulated [Ransohoff, 2005].

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