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Translational pathology of neoplasia

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Abstract

With the increasing use of individualized medical care (personalized medicine) in treating and managing patients with cancer, the utilization of biomarkers in selecting and tailoring such medical approaches also is increasing and becoming more important. Specifically, many therapies are effective against only a subgroup of a specific type of tumors and exposing patients with different non-responsive subgroups of the same tumor to ineffective therapies, not only exposes these patients needlessly to acute and chronic side effects of the therapy, but also adds to the costs of medical care. For example, the Oncotype Dx test for estrogen receptor positive tumors that are node negative has been used to identify low risk tumors for which surgery alone is an adequate therapy. Biomarkers may be used to aid in multiple aspects of medical care related to cancer, including early detection, diagnosis, risk assessment, as well as in predicting the aggressiveness of cancers (i.e., prognosis) and predicting the therapeutic efficacy of treatments (i.e., prediction). Biomarkers may be also used as surrogate endpoints to aid in evaluating therapies and preventive approaches. Types of biomarkers vary greatly and include histopathologic appearance, stage of the lesion, quantitative morphologic features, size of the lesion, metastatic pattern and extent of metastasis, as well as imaging and molecular features. The types of measurements of biomarkers also vary; for example, molecular features can be measured at the DNA, mRNA or protein levels as well as at regulatory levels (e.g., microRNA). The usefulness of each biomarker is limited by its sensitivity and specificity in fulfilling its role (e.g., in early detection) and the requirements of sensitivity and specificity to accomplish specific tasks are affected by multiple variables. For example, both very high specificity and sensitivity of a test are required to screen a population with a low prevalence of a specific tumor. The goal of this manuscript is to introduce the reader to how biomarkers may be used and the limitations on the uses of biomarkers in translational research.

Keywords

Sensitivity; specificity; early detection; prognosis; risk assessment; surrogate endpoints; diagnosis; receiver operating characteristic; prediction; biomarkers; prevalence; medical costs; side effects; histopathology; molecular features; imaging; prevention; treatment; personalized medicine; individualized medical care

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1. Introduction

Over the last 50 years of basic research, numerous genes, proteins, signal transduction pathways and other molecules (e.g., microRNA) have been identified that are differentially present in pre-invasive neoplasia and in cancers compared to normal tissues. In addition to the identification of the genes of the human genome, literally thousands of other modified proteins (e.g., phosphorylated or splice variants) also have been identified whose phenotypic expressions are modified in neoplasia and similar processes such as tissue repair or inflammation.

Translational research is research in which any of these molecules or molecular pathways are translated into being useful clinically, i.e., in practical medical uses that directly affect medical care. Such uses include aids in early detection, determination of clinical outcomes (prognosis), diagnosis, detection of recurrence after therapy, risk assessment, identification of targets for therapy, prediction of responses to therapies (i.e., prediction), monitoring clinical outcomes of therapies (i.e., surrogate endpoints), and imaging diseases processes [1–31]. These areas of translational research are discussed subsequently.

1.1. Early detection

It is very important to identify disease processes as rapidly as possible in order to limit the damage of the disease, to treat the disease at a stage at which the disease can be more easily managed and/or to diagnose the disease when it can be cured. The easiest stage to cure a neoplastic disease is at the stage of pre-invasive neoplasia [32]. If all and subsequent recurrent pre-invasive neoplasia (PINN) can be eliminated from a patient, then PINN will not become invasive and hence the neoplastic process will not become life threatening. For example, with the use of the PAP smear, pre-invasive squamous neoplastic lesions of the cervix (cervical intraepithelial neoplasia [CINs]) can be identified and removed or treated before the lesion invades; thus squamous cell carcinoma (SCC) of the cervix can be prevented in a society which screens and treats all women for high grade CIN. Because of the nature of some forms of PINN, the pre-invasive lesions may recur following successful therapy; however, the time course of the disease usually does not increase so the potential development of invasive disease would be delayed after each removal of PINN.

The goal of the translational pathology of PINN is the reliable identification of the lesion by the least invasive and most accurate methods possible. For example, in screening for CIN, originally the screening test was principally by histopathological examination of the cells removed during the scraping of the squamous columnar junction of the cervix. If CIN2 or CIN3 were detected by cytologic examination, then a colposcopic examination and biopsies of cervical lesions would dictate whether or not there was a need for further treatment or for careful follow-up. With the understanding of the biology of CIN, the over treatment of some CIN2 which might not progress and the viral origin of CIN, the medical evaluation of CIN now may include a measure of the types of human papilloma viruses (HPV) present in the lesion [32]. If strongly pathogenic HPVs are detected (e.g., HPV16, 18, 31, 33 or 45), the likelihood of the progression of CIN to SCC is greatly increased. HPV analysis can now identify 13 high risk types of HPV; an extensive study of HPV screening in India indicated that HPV screening identified more cases of cancer and reduced cancer deaths compared to cytologic screening [33]. Also, the understanding of the etiology of cervical cancer has led to vaccine therapy directed at specific pathologic strains of HPV, e.g., HPV 16 and 18 [34].

The screening for neoplastic processes other than cervix has followed different approaches. For example, radiological imaging of the breast by mammography has led to the detection of small lesions in the breast and hence the early identification of not only invasive mammary carcinoma, but also in situ carcinoma. Even with the success of the early detection of

cervical cancer and breast cancer in the industrially developed world, because of the costs of these methods of early detection, such screening has not been widely adopted in developing countries.

A major goal of early detection is a test of high sensitivity and specificity that can be performed on easily obtained bodily fluids/samples such as blood, urine, saliva, or feces. A major feature of an early detection test is its sensitivity and specificity. The sensitivity of the test should permit most cases of the neoplastic process to be identified while it is curable. Thus, an early detection test is of little use if it only can identify a large tumor burden which is primarily correlated with large invasive tumors or metastatic disease. For example, the soluble component erbB-2 (HER2/neu) or p105^{erbB-2} was found elevated primarily in more advanced cancers of the prostate [35] and breast. Also of great concern is the specificity of a test because the rate of false positives which are inversely related to the incidence of the neoplastic process, are very important, especially when the next step after the positive results is of great expense or morbidity.

Some of the major serum/plasma tests for diseases are listed in Table 1. Each of these tests has problems associated with its use as “screening tests” for the early detection of neoplasia [36]. For example, prostatic specific antigen (PSA) is produced by normal prostatic glandular cells and hence benign prostatic hyperplasia (BPH) also causes an elevation of PSA [37–39]. This especially is a problem because BPH causes elevations of PSA that are in the range 2.5–10 ng/ml – the early abnormal range of PSA in which the PSA marker would be most useful in early detection of prostatic cancer. Nevertheless, the use of PSA as a screening test has been adopted because the diagnosis of prostate cancer is so frequent in men over 50 years of age; as a consequence of an elevated PSA, a 12 core biopsy of the prostate is likely to identify prostate cancer. In contrast, the CA125 test is a relatively sensitive test for ovarian carcinoma; yet CA125 is not frequently used as a screening test for ovarian cancer in an asymptomatic, low risk population of women because of its specificity and the high rate of false positives that result because ovarian carcinoma is relatively uncommon [36].

The above issue is demonstrated as follows: If a test is 100% sensitive and 90% specific, but a tumor occurs in only one in 1000 patients, in testing 1000 patients the test will identify the

one patient with tumor (true positives (TP) positive e.g., $\frac{\text{true positives (TP)}}{\text{false negatives} + \text{TP}} = 1.0$), but also over 10% or 111 patients without tumor (false positives e.g., $\frac{\text{true negatives (TN)}}{\text{false positives} + \text{TN}} = 0.9$). In this case the issue on using the test in screening becomes the cost and morbidity associated with follow-up of each of the 111 false positive cases with a more specific test. Thus, the cost, morbidity of follow-up testing, and acceptability of testing as a screening test should be balanced with decreased morbidity and mortality of the disease based on using the screening test [36].

Another limitation of many biomarkers for the early detection of a specific neoplastic process is that many biomarkers are not specific for one type of tumor. For example, increases in serum levels of interleukin 6 (il-6), il-8 or CEA may occur in patients with many types of cancer (e.g., pancreatic or colorectal cancers) as well as some inflammatory processes. Thus an elevated level of a biomarker may indicate a potential tumor in multiple sites (e.g., lung and colon). This may be more likely when the elevation of the screening biomarker is secondary to a general immunological or tissue reaction to a tumor, rather than to a product released specifically by a tumor (38, Fig. 1). For example, acute phase reactants may be elevated secondary to the body’s reaction to a tumor rather than by being produced specifically by the tumor.

The challenge of early detection is to find a marker(s) that are both sensitive and specific and that can be detected, based on biomarker levels in bodily fluids or image/fine needle aspiration, when a neoplastic process is curable (i.e., when the neoplastic process is small or pre-invasive). Except for prostatic specific antigen, most biomarkers that have been reported are not tumor or organ specific. Because a single marker that is both sensitive and specific as well as organ specific has proved to be very difficult to identify for most organ systems or specific tumors, a combination of biomarkers may need to be used in early detection of specific tumors. In this combination of biomarkers, some biomarkers might add sensitivity and others specificity.

1.2. Detection of recurrence or metastatic disease

While not very useful in screening for initial disease, most of the molecules listed in Table 1 are very useful to detect the recurrence of tumors which have been surgically removed or treated with radiation. For example, when the prostate or the source of PSA has been removed either by surgery, successful radiation or hormonal therapy, all of which act on normal as well as neoplastic prostate tissue, PSA should drop to the minimum detectable range; thus, a subsequent consistent increase in PSA is indicative of recurrence or of metastatic disease. Similarly, a continued elevation of CA125 following removal of both ovaries or a subsequent rapid increase in CA125 is usually indicative of recurrence, metastatic ovarian carcinoma or rarely a different primary cancer, that may also produce CA125, for example, pancreatic cancer.

1.3. Diagnosis

The use of biomarkers in diagnosis is closely related to the use of biomarkers in early detection and/or to detect recurrence. The only difference between early detection and diagnosis may be the stage of the disease being detected or diagnosed and the accuracy of the detection. Thus, a method that is 100% sensitive and 100% specific for the early detection of a neoplastic process is actually a diagnostic method. The actual diagnosis of neoplastic lesions may be based upon measurement of biomarkers in either the tumor tissue or in bodily fluids. For example, biomarkers may be very useful in aiding the diagnosis of neoplasia when they are specifically expressed in the tissue of the neoplastic process. Depending upon the biomarker, such biomarkers may be identified by histochemical assays, immunological assays or by microchemical methods.

Over the last three decades, the use of immunohistochemistry to identify biomarkers useful in the diagnosis of neoplastic processes has revolutionized the practice of pathology with a major shift from subtyping tumors using histochemistry to using immunohistochemistry which is usually more specific and more sensitive than histochemistry. For example, melanomas were once diagnosed by using the Fontana-Masson histochemical stain which identifies by staining with a silver solution cells with Argentaffin characteristics. In contrast, currently many immunohistochemical stains which are more specific for melanomas such as MELAN A, HMB45 or S100 are used to diagnose melanomas.

Some biomarkers such as number of mitoses have also been used to separate malignant (e.g., leiomyosarcomas) from benign tumors (e.g., leiomyomas). Such a pathological separation is beyond a marker of prognosis which usually applies to separation of more aggressive from less aggressive cancers as will be discussed subsequently. Several immunohistochemical markers can substitute for counting mitoses (Ki67, PCNA) or may aid in counting mitoses.

In some cases with limited material (small biopsies with a few foci of atypical cells), biomarkers may be used as an aid to separate malignant from benign lesions. For example,

in prostate and breast, the presence of basal cells may separate malignant from benign glands because malignant glands typically do not have both luminal and basal cells. In breast, S100 and smooth muscle actin may be used to identify basal myoepithelial cells. In the case of the prostate, basal cells are stained with a basal cytokeratin (34 β E12) or p63. Similarly, new markers such as α -methylacyl-CoA-racemase (AMACR) may be expressed in a proportion of malignant cells of the prostate [42] but not in normal or uninvolved prostate cells.

Recently, biomarkers have been increasingly used to subdivide or subtype tumors such as breast carcinomas. By the original classification of breast carcinomas by histopathological analysis several main subtypes were characterized including ductal, lobular, medullary, tubular and papillary. The molecular analysis of breast cancer has further subdivided the above lesions based on molecular expression [43–50]. The luminal type of breast cancer has been characterized primarily by ER⁺ cells and this group was subdivided into Luminal Type A, B and C. In contrast, breast cancer with ER⁻ cells has been subclassified as of basal type (1 and 2), HER2/neu (erbB-2) subtype, or a triple negative subtype. Of interest, there is significant overlap between basal type (BT) and triple negative breast cancers (TN) with perhaps 28% of TN as being non-basal subtype and 23% of the basal type being non-TN. There also is a strong overlap of both groups with some type of dysregulation in BRCA-1 [48–50]. See Fig. 2. Of interest only medullary carcinoma is associated with primarily one molecular phenotype, the basal cell subtype.

Another use of biomarkers in diagnosis is the identification by biomarker expression of the sources of metastases when a primary cancer has not been identified. In some cases, the marker, such as prostatic specific antigen (PSA), is very accurate in identifying metastatic foci of prostate cancers in tissues. In other cases, the demographics of the patient, the pattern of metastases (e.g., in bone) plus the expression of biomarker is useful. For example, an undifferentiated epithelial-like cancer in the axillary lymph nodes of a woman is a melanoma if the cancer expresses MELAN A, but is likely a breast cancer if the lesion does not express MELAN A, HMB45, or S100, but instead expresses generalized mixtures of high and low weight keratins such as AE1/AE3. Alternatively, the lesion may be a lymphoma if it expresses CD3 or CD20. A general molecular approach to characterizing a metastatic lesion is depicted in Fig. 3.

Thus, to determine the source of a metastatic lesion, the pathologist and oncologist must rely on the history of the patient as to prior malignant lesions. If none, then the demographics of the patient and the pattern of metastases are important in identifying the primary lesion. For example, metastases only to local lymph nodes and especially to a sentinel node is usually indicative of the location of a lesion draining to these nodes. Finally, each type of cancer has a typical pattern of metastatic spread. For example, gastrointestinal carcinomas metastasize to local lymph nodes and then to the liver, while prostate cancer metastasizes to local lymph nodes and then to bones, especially the vertebral column. After the clues provided by the sex and age of the patient and the pattern of metastases, the pathologist and oncologist frequently must rely on molecular markers to confirm the primary tumor.

Biochemical markers in serum/plasma can also be used in diagnosis of neoplasia. For example, small adrenal cortical tumors producing primarily cortisol and the clinical picture of pure Cushing's syndrome are likely benign; however, an adrenal cortical tumor producing excessive androgens is likely malignant. Similarly, a small paraganglioma of the adrenal gland producing primarily norepinephrine is more likely to be benign than a similar paraganglioma of the same site producing primarily dopamine; paragangliomas at other sites (e.g., paravertebral) may have a greater rate of malignancy. Also, the identification of a paraprotein of 10 g/L in serum may be indicative of a plasmacytoma or multiple myeloma,

but a paraprotein of < 10 g/L may be indicative of a process that may not progress to a plasmacytic malignancy or to a lymphoma.

1.4. Surrogate endpoint biomarkers

Evaluation of some clinical endpoints such as the prevention of malignancy by a chemopreventive agent may take several years and involve thousands of patients in order to complete the analysis. Thus, such testing can be prohibitively expensive when many agents must be evaluated. If a biomarker(s) could be identified for which changes in the biomarker(s) correlated exactly with a preventive response of an agent, then changes in such biomarker(s) could be used as surrogates to the clinical response; such markers are called surrogate endpoint biomarkers (SEBs). If a biomarker always changed many months to years before a preventive response, then the biomarker could be used as a SEB, i.e., as an inexpensive screening method to determine the effectiveness of preventive agents; then if SEBs indicated that a preventive agent was likely to be successful, the preventive agent could be evaluated by treating a population in which changes in SEBs could be correlated with partial or complete resolution of PINN. Thus, exhaustive clinical trials could be undertaken only for those agents that were most promising in initial trials. It is important to understand that the SEBs should correlate with clinical prevention of the lesion (e.g., complete or partial resolution of a preinvasive neoplastic lesion) and not be only correlated with administration of the clinical drug. Consider Fig. 4.

An example of such a preventive therapeutic approach is the testing of “preventive agents” (i.e., agents that prevent a malignant lesion from developing in a high risk population or which reverses pre-invasive neoplastic lesions which have developed in an at risk population). For example, a preventive agent may cause the resolution or prevention of dysplastic leukoplakia in abusers of tobacco [15,51], hence the prevention of the development of squamous cell carcinoma of the oral cavity.

1.5. Determination of clinical outcome or prognosis

Besides the diagnosis or subtype of a neoplastic process, many efforts have been made to determine the clinical aggressiveness of malignant lesions. These efforts to categorize the aggressiveness of cancers began with determining the stage and grade of cancers. This was the original basis for the staging of cancers; however, with the utilization of molecular analysis, currently stage is not always the controlling variable in determining clinical outcome [52]. In the case of prostate cancer, Gleason developed a score based on histopathologic features that ranged from 1 to 5 for a single focus of adenocarcinoma or 2 to 10 based upon both the primary and secondary patterns of cancer growth. Low scoring cancers (Gleason combined score 6) usually have indolent courses, high scoring cancers (score 8) usually have aggressive outcomes while intermediate scoring cancers, Gleason 7, cannot be easily predicted as to their aggressiveness. Similarly in breast cancer, the modified Bloom and Richardson grading method as well as cytomorphometric analysis have both proved useful in identifying aggressive subtypes of ductal carcinomas [53]. For many cancers, the grade of the cells of the cancer is quite important in outcome.

As discussed, biomarkers have recently been identified that are used in subtyping carcinomas based upon the extent of expression of multiple biomarkers. Specifically, the ER⁺ Type 1 subtype of breast cancer has been identified as having an excellent outcome; in contrast, breast cancers that do not express estrogen receptor, progesterone receptor and p185^{erbB-2} have a poor outcome even though a cancer with high p185^{erbB-2}, if untreated, has a poor outcome. For treated lesions, the lack of expression of p185^{erbB-2} takes away the option of therapy with Herceptin [43–47]. Also, our results in colorectal cancer in proximal carcinomas in Caucasian-Americans indicate those cancers expressing Bcl-2 (high),

p27^{Kip-1} (high) and no nuclear accumulation of p53 have relatively good outcomes [16,20,28].

Epigenetic changes also may affect the aggressiveness of neoplastic processes. As we begin to understand more about epigenetic changes and the development of cancer, increased molecular changes associated with epigenetics have been associated with subsets of cancers that are more or less aggressive. Some epigenetic changes such as methylation of CpG islands associated with the aggressiveness of cancers depend on increased expression of DNA methyl transferase in prostate cancer and increased histone methyl transferase such as EZH2 in breast and prostate cancers [54].

The characteristics of most malignant processes may vary with race and ethnic background. For example, the incidence and aggressiveness of prostate cancer is increased in African-Americans (AA) compared to Caucasian Americans (CA), but the incidence in Hispanic Americans (HA) is less than CA. The usefulness of using specific biomarkers for predicting the aggressiveness of tumors also may vary with race. For example, in colon adenocarcinoma, the nuclear accumulation of p53 as determined by immunohistochemistry is a strong prognostic biomarker for proximal colonic carcinoma, but p53 is not a useful prognostic biomarker in AAs [28]; for African-Americans, malignant cells in colorectal cancer with high grade are a prognostic indicator of very poor outcome [5]. Similarly, biomarkers of clinical outcome of breast cancer have been reported to vary with race [9,10,55].

1.6. Risk assessment

Patients whose genotypes (germ line DNA) carry specific mutations are at risk for developing specific types of cancers. If a risk of developing a cancer is high, then screening methods using biomarkers may be useful even when these same screening methods are a waste of resources for patients with lower risk. For example, children and adults who are from families with multiple endocrine neoplasia Type 2 (MEN2) and carry a mutation in the RET oncogene may be monitored for the occurrence of medullary carcinoma of the thyroid by periodically measuring calcitonin. In contrast, adults carrying a germ line mutation in enzymes that repair DNA mismatches (e.g., MLH2 and MSH6) have an increased risk for several neoplastic processes, especially adenocarcinomas of the proximal colon as do patients that carry a mutated copy of the APC gene. Besides germ line mutations which alone carry an increased risk for the development of specific neoplastic processes, certain other inherited genes result in an increased risk of developing specific cancers when combined with environmental exposures. For example, in a heavy smoker who has a glutathione S-transferase polymorphism in which GSTM1 is reduced has an increased risk for lung cancer [56]. Similar variations in acetylation ability secondary to variants of N-acetyltransferase (NAT) not only affect drug metabolism and hence the effectiveness and toxicity of specific drugs, but also increase the risk for some cancers, especially urothelial cancers of the bladder [57].

Similarly, epigenetic changes in the methylation of specific genes at CpG islands have major effects in the development of early neoplastic changes. Frequently, the methylated CpG islands involve important regulatory elements of suppressor genes such as p16 and of those genes involved in mismatch repair such as MLH1, in apoptosis, cell adhesion (E-cadherin) and in cellular migration (TIMPs); such patterns of methylation result in increased risk for specific cancers.

1.7. Targets for novel therapies

As our understanding of various pathways of cellular signaling and function have expanded and our knowledge of how these pathways are involved in the development and progression of neoplasia, important steps in these pathways are now targeted as primary or secondary forms of therapy for specific neoplastic processes. One of the initial targets was p185^{erbB-2} or HER2/neu. A monoclonal antibody was developed against p185^{erbB-2} (Trastuzumab or Herceptin) and it was found to inhibit effectively the functioning of p185^{erbB-2} and to be effective against breast cancers (and subsequently other cancers) that over-express genes coding for p185^{erbB-2}. Therapy with Herceptin is also now approved for treating breast cancers which overexpresses p185^{erbB-2}.

The EGFr pathway can similarly be targeted by antibodies to EGFr (e.g., Cetuximab). Because almost all SCCs of the oral cavity express large amounts of EGFr on their cell surface membranes, Cetuximab has proved very effective in combined therapy with radiation in squamous lesions of the oral cavity [58]. Antibodies to EGFr have also been evaluated as therapeutic agents for cancers of the lung. They have proved primarily effective in only a small proportion of lung adenocarcinomas (especially bronchoalveolar type, now designated a form of intraepithelial neoplasia leading to adenocarcinoma of the lung) that have activating mutations or overexpression of EGFr [59,60]. In addition, multiple monoclonal antibodies have been developed to target specific molecules or processes e.g., inflammation, thought to provide a stimulating input to various cancers or to the development of the vasculature of cancers (Table 2) and these and many additional such antibodies are now in various stages of testing for a wide range of tumors [7,8,12,14].

Other molecules, many of which are designated small (inhibitory) molecules (e.g., < 50D in molecular weight) have been developed to target the functional aspects of endogenous molecules or pathways thought to stimulate the growth of cancers. For example, gefitinib (Iressa) was developed to target the phosphokinase functions of EGFr; however, when tested as to its effectiveness against non-small cell lung cancers (NSCLC), like cetuximab, it required a tyrosine-kinase mutation in EGFr and/or some dysregulation or overexpression of EGFr expression for the therapy to be successful. However, a similar phosphokinase inhibitor of EGFr, erlotinib (Tarceva) has been approved for treatment of NSCLC.

Another small molecule developed to target a transduction system is Gleevec which targets the tyrosine-kinase domain of the fusion protein bcr:abl and the ATP binding pocket of KIT and PDGFRA. CML which frequently has the fusion protein bcr:abl is usually responsive to imatinib or the related kinase inhibitor dasatinib. Also, neoplastic lesions, especially gastrointestinal stromal tumors (GIST), with mutations in KIT or PDGFRA are usually at least transiently responsive to imatinib.

As described above, targeting of growth factors or their receptors with monoclonal antibodies or using small molecules to inhibit signal transduction pathways of growth factors aids in therapy of some neoplastic processes. Table 3 lists small inhibitory molecules that target signal transduction pathways.

Thus, translational research to identify therapeutic approaches which target signal transduction pathways which modulate specific neoplastic processes has been successful for multiple types of tumors. This area of translational research is rapidly expanding and is extremely promising.

1.8. Patterns of molecular expression to predict therapeutic responsiveness (prediction)

An important goal of translational pathology is to be able to use molecular features of a type of neoplasia to predict its responsiveness to specific therapies. Obviously, if the therapy

targets a known biological feature, the presence of that biological feature is usually needed for successful therapy. For example, proliferation may be turned on and targeted to be turned off or apoptosis may be turned off and targeted to turn on. Similarly, if a specific molecule or pathway is targeted by a therapy, the presence of that molecule at a specific level may be necessary for the therapy to be successful. Sometimes the molecule targeted must be “turned-on” or constitutively active for the therapy to be successful, such as EGFR in the therapy for NSCLC; however, rarely some therapy such as therapy with the TRA-8 antibody to the death receptor, DR5, is not based in this case on target levels of DR5 [12,14]. Thus, some types of therapy may work independent of high levels of the molecules which are thought to be targets of the therapy. In such cases, it would be useful to be able to identify molecular features which can be correlated with the potential effectiveness of a therapy. This area of translational research is very early in its development, but it is potentially very important.

1.9. Imaging

Techniques which image neoplastic lesions have improved greatly over the last three decades, especially in the modalities of imaging and the sensitivity of imaging, including the minimum size of lesions that can be imaged. Imaging, including x-ray computerized tomography (CT), magnetic resonance imaging (MRI), ultrasound (US), positron emission tomography (PET), single-photon emission computerized tomography (SPET), and bioluminescence and fluorescence optical imaging, can be used successfully in identifying primary or metastatic lesions (i.e., staging of disease) and to separate benign from malignant lesions. These imaging models, e.g., ultrasound, can be used to detect neoplastic nodules and hence guide fine needles for aspiration and thus to aid in early detection/diagnosis [11]. Other uses of imaging methods include determining the effectiveness of specific therapies, evaluating the pharmacokinetics of therapeutic drugs, predicting drug resistance, determining tumor vascularity of neoplastic lesions, evaluating novel therapies in animal models and aids in evaluating gene therapy [7,8,61,62]. Imaging can be used to evaluate neoplastic processes including the evaluation of the extent of tumor metabolism using labeled glucose transport analogues. Also agents for imaging that correlate with proliferation and apoptosis are under development. In general, imaging, although a relatively new area of translational research, is a major growth area, as many of the clinical targets for therapy can also be targeted for imaging.

2. Challenges and advances in translational pathology

Several areas of translational pathology use immunohistochemistry in evaluations. For example, the determination of estrogen receptor and progesterone receptors in the diagnosis/prognosis of breast cancers as well as erbB-2 expression to verify the target for Herceptin therapy can be measured by this technique. The collection and processing of tissues can have a great effect on immunohistochemistry [63]; yet the effects on immunohistochemistry of collection and processing of tissues are inadequately understood. Specifically both the fixation and the processing of tissues to paraffin blocks may greatly affect immunohistochemistry [64, 65]. In addition, standardized methods of performing immunohistochemistry have not been accepted [66]. For example, some antigens (e.g., pAkt) rapidly decay when exposed to ischemia while the phenotypic expression of other proteins (e.g., heat shock proteins) may be increased by warm ischemia (reviewed in [67]).

Over the years there has been a great concern that the degradation of mRNA will be limiting in the use of human tissues to identify molecular features of neoplastic lesions. Our current knowledge indicates that the more unstable types of mRNA are most likely degraded during the period of warm ischemia, i.e., between compromise of the vascular supply to an area of tissue and the removal of the tissue. One of the advances in evaluating translational

pathology is the use of amplicons of < 100 bp in the analysis of gene expression using real time quantitative PCR [68,69]. Specifically, our results and those of others indicate that the use of small amplicons permits the successful utilization of partially degraded mRNA in studies of gene expression in tissues that have extended periods of warm ischemia. As the speed of complete sequencing of genes increases as its costs decrease, full sequencing is likely to replace PCR in analysis. Of concern will be the quality of the mRNA and the effects of partial degradation of mRNA on the results of sequencing. These issues are now being evaluated.

In summary, evaluating the translational pathology of neoplastic processes has been greatly slowed by a lack of understanding of the effects of handling of human tissues on molecular methods as well as attention to the quality of human tissues used in assays. In addition, the methodology used in analysis has not been standardized. This creates problems in the literature, especially for molecules for which there are multiple splice variants and different patterns of staining depending if antibodies are to the C or N tail of the antigen.

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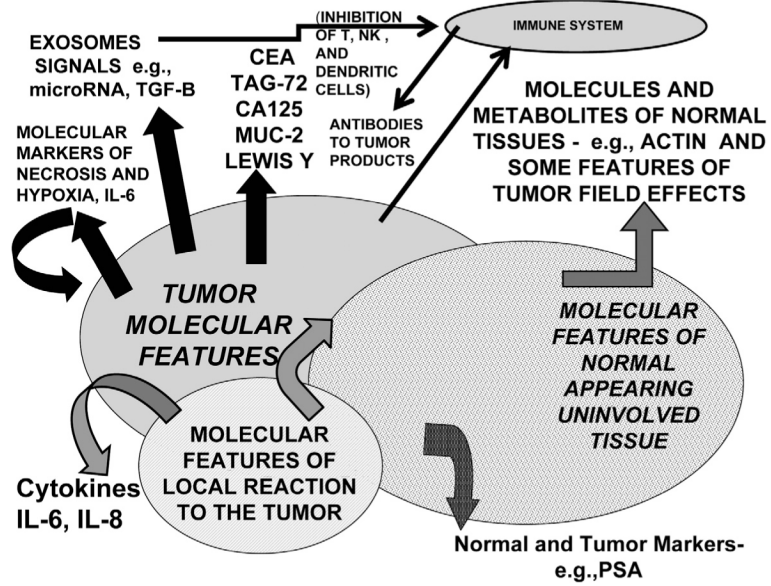


Fig. 1. Demonstrates the complex interaction between a tumor, its surround and the immune system. It shows how various biomarkers may be associated with the presence of a tumor without coming directly or uniquely from a tumor. For example, PSA is produced at higher levels in the cells of normal glandular epithelia of the prostate than in cells of prostate cancer.

Molecular Characterization of Breast Cancers – All Types

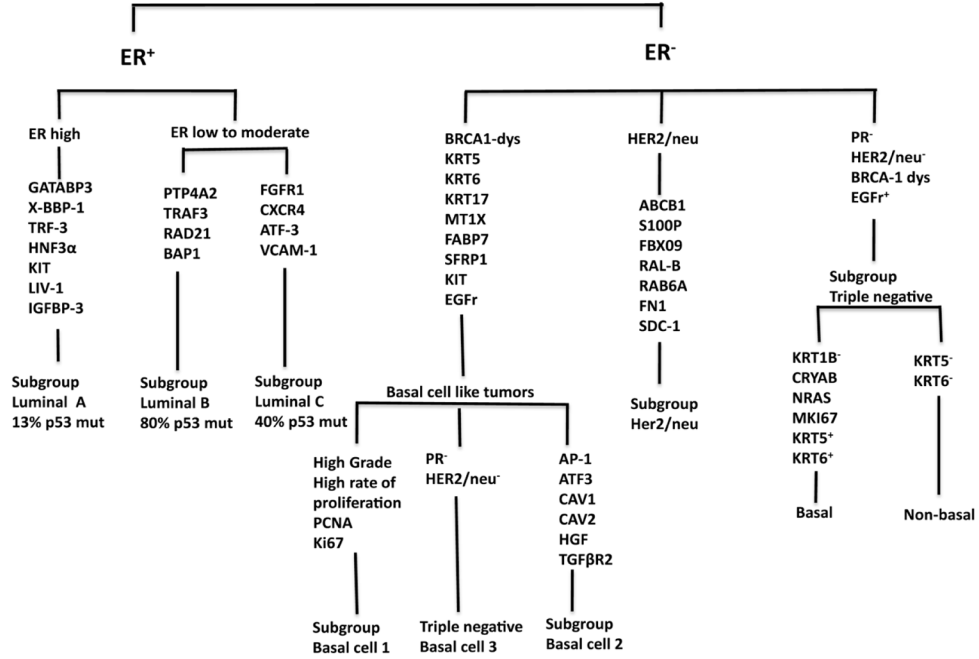


Fig. 2. Demonstrates the molecular subtyping of breast carcinoma. The abbreviations used are defined subsequently. (GATABP-3) GATA binding protein 3; (X-BBP-1) box binding protein 1; (TRF-3) trefoil factor 3; (HNF3 α) hepatocyte nuclear factor – 3 α ; (PTP4A2) protein tyrosine phosphatase type IVA member; (TRAF3) Tumor necrosis factor receptor associated factor 3; (BAP-1) BRCA associated protein 1; (KPT5, -6 or -17) keratin 5, 6 or 17; (MTIX) metaiothionein 1x; (FABP7) fatty acid binding protein 7; (SFRP1) frizzled related protein 1; (ATF3) activating transcription factor 3; (CAV1 or 2) caveolin 1 and 2; (HGF) hepatocyte growth factor; (TGF β R2) transforming growth factor β receptor II; (ABCB1) multidrug resistance protein 1; (S100P) S100 calcium binding protein P; (FBX09) fatty acid syntase; (RALB) GTP binding protein; (RAB6A) member of RAS oncogene family; (FN1) fibronectin 1; (SDC1) syndecan 1. In the future, the complex cancer such as lung or renal carcinomas also are likely to be subdivided molecularly.

Initial Identification of an Undifferentiated Malignant Tumor

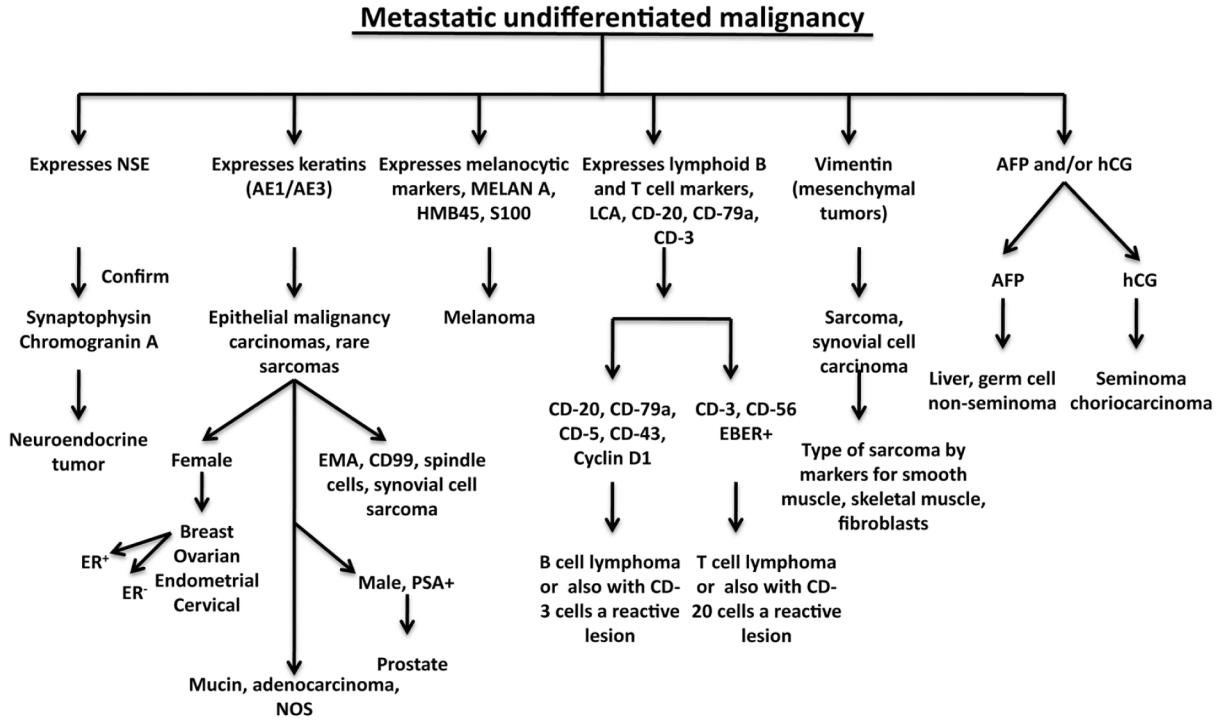


Fig. 3. Demonstrates how molecular analysis aids in identifying poorly differentiated malignant tumors, especially metastatic cancers, whose primary cancer is unknown. Typically the pathologist may suspect which type of tumor is the primary cancer and only a few of these molecular markers are used to confirm the initial impression.

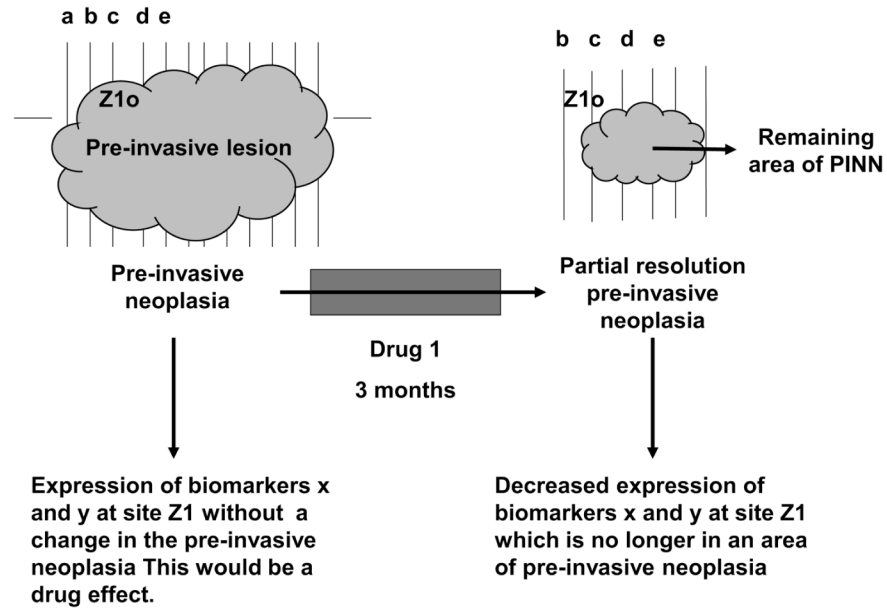


Fig. 4.

In Fig. 4, note biomarkers x and y may change at site Z1 without the resolution of the area of pre-invasive neoplasia. This would correlate with an effect of the drug and not a pattern indicative of a surrogate endpoint. Similarly, areas of pre-invasive neoplasia may “move” spontaneously, with or without resolution of the extent of the PINN. In this case, there is partial resolution of the area of PINN. The movements of PINN lesions over a period of months without even partial resolution of the PINN is why the use of “controls” is so important in studies to identify responses in SEBs to drugs used in the prevention of neoplasia [51].

Table 1

Serum/plasma biomarkers in neoplasia

Serum/plasma marker	Source malignant lesion
PSA	Prostatic adenocarcinoma
CA125 (MUC 16)	Female – ovarian, epithelial cancer; GI cancers; endometrial cancers
CA19.9	Pancreatic carcinoma
CA15-3, CA27.29 (MUC 1)	Prognosis of breast cancer; response to RX of breast cancer
CEA, CEACAM-1	Gastrointestinal carcinoma, pancreatic carcinoma
AFP	Hepatocellular carcinoma, germ cell, nonseminoma
hCG	Germ cell – seminomas; choriocarcinoma
p105 ^{erbB-2}	Breast, prostate, ovarian cancers
Long DNA	Many types of cancer, e.g., in feces, colorectal cancer
TPA *	Many types of cancers
Circulating tumor cells (CTC)	All cases of cancer

* Tissue polypeptide antigen (TPA) is a heterogenous combination of cytokeratins 8, 18, and 19 that can be measured in serum or urine; TPA has been considered as markers of cellular proliferation or apoptosis and hence maybe increased in patients with tumors [40,41].

Table 2

Monoclonal antibodies used to target signal pathways in cancer

Molecular target	Name	Commercial name	Cancers targeted	Comments
EGFr	Cetuximab	Erbitux	Oral, non-small cell lung	Non-small cell carcinoma of the lung with EGFr activation. Oral cancer together with radiation
Her-2/neu	Trastuzumab	Herceptin	Breast	
VEGF	Bevacizumab	Avastin	Colorectal; breast	
CD20	Rituximab	Rituxan	Lymphoid	
CD20	Ibritumomab tiuxetan * Rituximab	Zevalin	B cell lymphoma	
CD26	Tositumomab **	Bexxar	B cell lymphoma	
CD33	Gemtuzumab ozogamicin (monoclonal anti-body combined with caicheamicin, an antitumor antibiotic)	Mylotary	Acute myelogenous leukemia	
CD52	Alemtuzumab	Campath	Chronic lymphoid leukemia	

* Frequently labeled with Y-90;

** Labeled with I-131.

Table 3

Small molecules/specific molecules used to target aspects of signal pathways in cancer

Signal transduction pathway target	Therapeutic small molecule	Commercial name	Molecular target	Cancer targeted	Comments
EGFr	Gefitinib	Iressa	Tyrosine kinase – EGFr	Bronchoalveolar carcinoma in Japanese women	Only activated EGFr; not on market
EGFr	Erlotinib	Tarceva	Tyrosine kinase – EGFr	Non-small cell lung/pancreas	In combination with gemcitabine for pancreas
Kit, PDGFR, Ber:abl	Imatinib	Gleevec	Tyrosine kinase of c kit and PDGFR; fusion protein, ber:abl	GIST, CML	Response is frequently transient
Ber:abl, SRC	Dasatinib	Sprycel	Tyrosine kinase	CML	Works on some patients resistant to imatinib
VEGFR – 2 & 3, FLT-3, Kit, PDGFR- β , Fms, craf, braf	Sorafenib	Nexavar	Tyrosine kinase of target pathway	Renal	
PDGFR- α & β , VEG-FR 1, 2, 3, Kit, FLT-3, CSF-1, RET	sunitinib	Sutent	Multiple kinases of pathway	GIST, renal	
GnRH	Abarelix	Plenaxis	Antagonist to GnRH receptor	Breast and prostate	
CD25	denileukin; diftitox	Ontak	CD25 component of il-2 receptor	Cutaneous T cell lymphoma	
CXCR1	Repertaxin	Repertaxin	il-8 receptor	All	Reduces inflammation mediated by il-8