

Predictive Markers in Breast and Other Cancers: A Review

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Background: Unpredictable efficacy and toxicity are hallmarks of most anticancer therapies. Predictive markers are factors that are associated with response or resistance to a particular therapy.

Methods: The English literature relating to predictive markers in oncology was reviewed. Particular attention was paid to metaanalyses, systematic reviews, prospective trials, and guidelines issued by expert panels.

Results: The prototype predictive tests in oncology are the estrogen receptor (ER) and progesterone receptor (PR), which are used to select patients with breast cancer likely to respond to hormone therapy. A more recently introduced predictive marker is HER-2 for selecting patients with advanced breast cancer for treatment with the therapeutic antibody trastuzumab (Herceptin). In adjuvant breast cancer, overproduction of HER-2 may also indicate an enhanced sensitivity to high-dose anthracycline-based regimens. On the other hand, in both early and advanced breast cancer, high concentrations of HER-2 appear to correlate with a lower probability of response to hormone therapy. Although many different anticancer drugs appear to mediate tumor regression by inducing apoptosis, there is currently no consistent evidence that any of the molecules implicated in this process can be used as predictive markers.

Conclusions: Currently, the only recommended predictive markers in oncology are ER and PR for selecting endocrine-sensitive breast cancers and HER-2 for identifying breast cancer patients with metastatic disease who may benefit from trastuzumab. For malignancies other than breast cancers, validated predictive markers do not exist at present.

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A predictive marker can be defined as a factor that indicates sensitivity or resistance to a specific treatment. Predictive markers are important in oncology as different cancers vary widely in their response to particular therapies. Thus, for any specific type of cancer, only a proportion of patients will respond to a particular treatment (Table 1) (1–4), whereas most are likely to suffer from adverse side effects. For optimum patient management, it is therefore desirable to know in advance the likelihood of a tumor responding to the therapy under consideration.

Predictive markers are sometimes confused with prognostic markers. Both types of markers are used to provide information on the likely future behavior of a tumor, but whereas predictive factors are used to prospectively select responsiveness or resistance to a specific treatment, prognostic factors provide information on outcome independent of systemic adjuvant therapy. Some markers can have both prognostic and predictive utility. For example, the estrogen receptor (ER)¹ in breast cancer not only predicts response to endocrine therapy but also correlates with good prognosis, at least in the short term. Whereas the use of markers for assessing prognosis has been widely discussed in recent years (5–7), there are few comprehensive reviews on predictive factors. The aim of this review is therefore to provide an overview on the current status of predictive markers in oncology. Because most work on predictive markers has been carried out on breast cancer, the main, but not exclusive, focus will be on this malignancy. The most widely studied predictive markers in oncology are now reviewed.

Hormone Receptors

CHEMISTRY AND BIOLOGY

The two most widely used predictive factors in cancer are the ER and the progesterone receptor (PR). Both the ER

¹ Nonstandard abbreviations: ER, estrogen receptor; PR, progesterone receptor; ASCO, American Society of Clinical Oncology; FISH, fluorescent in situ hybridization; CMF, cyclophosphamide, methotrexate, and 5-fluorouracil; 5-FU, 5-fluorouracil; ABC, ATP-binding cassette; TS, thymidylate synthase; and ALL, acute lymphoblastic leukemia.

Table 1. Approximate response rates of different advanced cancers to commonly used therapies.

Cancer	Treatment	Response, %	Ref.
Breast	Hormone	30	(1)
Breast	FAC ^a	50–80	(1)
Ovarian	Taxane-platinum	60	(2)
Testicular	Platinum regime	80	(3)
Colorectal	5FU + LV	20	(4)

^a FAC, fluorouracil, doxorubicin, and cyclophosphamide; 5FU + LV, 5-fluorouracil + leucovorin.

and PR are ligand-activated transcription factors belonging to the family of nuclear hormone receptors [for a review, see Ref. (8)]. Nuclear hormone receptors have several common structural features. These include a central DNA-binding domain responsible for targeting the receptors to specific DNA sequences within regulatory regions of their target genes and a ligand-binding domain, located in the carboxyl-terminal half of the receptor, that recognizes specific hormone and nonhormone ligands (8).

Both the ER and PR exist in two main forms. For the ER, these are known as ER α and ER β . ER α and ER β are the products of distinct genes but possess ~95% and 60% homology in their DNA- and ligand-binding domains, respectively (9).

Considerable divergence exists at the amino terminus with <25% homology (8). Both forms of receptor bind to the same DNA response elements and exhibit similar, but not identical, ligand-binding characteristics. In certain situations, ER β can attenuate the actions of ER α (9). For clinical purposes, only ER α is currently measured.

The two forms of PR, termed PR-A and PR-B, are transcribed from a single gene under the control of separate promoters (10). The main structural difference between PR-A and PR-B is that the A form lacks the first 164 amino-terminal amino acids contained in PR-B (10). Both forms of PR bind progesterins and interact with the PR-responsive element (10). A functional difference between PR-A and PR-B is that PR-A can act as a dominant repressor of both PR-B and ER in a promoter- and cell type-specific manner (11, 12).

USE OF ER AND PR FOR PREDICTING RESPONSE TO HORMONE THERAPY IN BREAST CANCER

Hormone therapy has been a mainstay of breast cancer treatment for more than 50 years. Initially, oophorectomy for premenopausal patients and pharmacologic concentrations of estrogens were used. More recently, these therapies have been replaced with antiestrogens (e.g., tamoxifen), aromatase inhibitors (e.g., anastrozole and letrozole), and luteinizing hormone-releasing hormone agonists (e.g., goserelin); for a review, see Ref. (13). Irrespective of the type of hormone therapy used, only ~30% of unselected patients with metastatic breast cancer respond (13).

Research carried out in the early 1970s showed that the

ER protein was present in 50–70% of invasive breast cancers. On the basis of a pooled analysis of ~400 patients with advanced breast cancer from eight different institutions, McGuire et al. (14) showed that 50–60% of women possessing ER-positive tumors responded to endocrine therapy. In contrast, only 5–10% of ER-negative tumors regressed with this treatment (14). It was later shown that 70–80% of breast cancers containing both ER and PR regressed with hormone therapy (15).

As well as predicting response to hormone therapy in advanced breast cancer, ER and PR are also associated with benefit from adjuvant endocrine treatment (16).

The relationship between steroid receptors and response to adjuvant tamoxifen was clearly shown in a metaanalysis involving more than 37 000 women with operable breast cancer enrolled in 55 randomized trials comparing tamoxifen vs placebo for the adjuvant treatment of breast cancer (16). The metaanalysis showed that adjuvant tamoxifen prolonged both disease-free and overall survival in patients with ER-rich tumors but had little benefit in patients who had ER-poor cancers (Table 2) (16). Although PR was assayed on fewer tumors than ER, knowledge of PR status did not appear to enhance the predictive power of ER (16). However, patients who were ER-negative but PR-positive did benefit from tamoxifen (16).

In contrast to findings from the metaanalysis (16), Bardou et al. (17), using results from two large databases, recently showed that the combined measurement of ER and PR is superior to ER alone in predicting benefit from adjuvant hormone therapy. The ability of PR to enhance the predictive potential of ER in this more recent study (17) may be attributable to the fact that all PR assays were carried out in two central laboratories using identical assays, whereas in the metaanalysis (16), PR assays were carried out in many different laboratories using different assays.

The contribution of PR to ER may also depend on the relative amounts of the two forms of PR present. For example, Hopp et al. (18) reported that patients with high PR-A:PR-B ratios in their breast cancers responded poorly to adjuvant therapy. This finding, if confirmed, would necessitate measurement of the individual forms of PR rather than total PR, which is the form measured with the currently available assays.

Table 2. Proportional decrease in recurrences and mortality with adjuvant tamoxifen in patients with ER-positive (ER+) and ER-negative (ER-) tumors.

Tamoxifen treatment	Recurrence, %		Mortality, %	
	ER+	ER-	ER+	ER-
1 year	21	6	14	6
2 years	28	13	18	7
5 years	50	6	28	-3

^a Data summarized from Ref. (16).

Whether ER β correlates with response or resistance to hormone therapy is currently unknown. As with ER α , tamoxifen and its active metabolite 4-OH-tamoxifen both bind to ER β and prevent estrogen-mediated transactivation at estrogen response elements (8). ER β is produced in a subset (40–70%) of invasive breast cancers (19). A preliminary report showed that ER β was produced in higher amounts in tamoxifen-resistant than in tamoxifen-sensitive cancers (20). In another preliminary report, however, the production of ER β was found to be associated with a favorable response to adjuvant tamoxifen therapy (21). Clearly, further work is necessary to establish whether ER β can prospectively predict resistance or response to hormone therapy in breast cancer.

Because of the striking difference in response of steroid receptor-positive and -negative breast cancers to hormone therapy, multiple expert panels, including an American Society of Clinical Oncology (ASCO) Expert Panel, the National Academy of Clinical Biochemistry (United States), a National Institutes of Health panel, the European Group on Tumor Markers, and the European Society of Mastology have recommended that ER (i.e., ER α) and PR be assayed on all primary breast cancers (22–26).

Currently, most investigators use immunohistochemistry to measure ER and PR. Unlike the older biochemical assays, immunohistochemical assays can be carried out on small tumors, including core needle biopsy material. Immunohistochemistry, however, is difficult to standardize, and assessment of staining score is subjective. According to Harvey et al. (27), patients with breast cancers containing as few as 1–10% of cells staining for ER respond to hormone therapy.

HER-2

CHEMISTRY AND BIOLOGY

The HER-2 protein, which is also known as c-erbB-2 or *neu*, is a member of subclass 1 of the superfamily of receptor tyrosine kinases. Other members of this family include epidermal growth factor receptor (HER-1), HER-3, and HER-4. All of these proteins possess an extracellular ligand-binding domain, a membrane-spanning region, and a cytoplasmic domain with tyrosine kinase activity [for a review, see Ref. (28)]. Although these receptors share a common structure, naturally occurring ligands have been discovered only for HER-1, HER-3, and HER-4. HER-2 thus appears to be an orphan receptor because no directly binding ligand has as yet been identified for it. HER-2, however, can signal as a result of heterodimerization with other HER family members and appears to be the preferred heterodimerization partner (28). After heterodimerization, HER-2 complexes initiate intracellular signaling via the mitogen-activated protein kinase, phosphatidylinositol 3'-kinase, and phospholipase C pathways (28).

In breast cell lines and model tumor systems, overexpression of the HER-2 gene has been associated with increased mitogenesis, malignant transformation, in-

creased cell motility, invasion, and metastasis (28). In human breast cancer, amplification of the HER-2 gene is found in 15–30% of primary invasive tumors. This means that instead of having only 2 copies of the gene per cell, up to 100 copies may be present. This increased gene copy number can lead to an increase in the number of receptors per cell from 20 000–50 000 up to 2 million (29). Either gene amplification or increased production of HER-2 is generally found to correlate with adverse prognosis, particularly in node-positive breast cancer patients (30).

Because HER-2 is involved in the pathogenesis and progression of certain breast cancers, exhibits extracellular accessibility, and is overexpressed in some cancers, it is a logical target for tumor-specific therapies. In particular, several monoclonal antibodies directed against the HER-2 ectodomain that specifically inhibit the growth of cell lines overexpressing HER-2 have been developed. One of these, known as 4D5, was modified for administration to patients by insertion of its complementarity determinant region into the structure of a consensus human IgG molecule. The resulting antibody was termed trastuzumab (HerceptinTM; Genentech Inc.) (31).

Trastuzumab was found to bind to HER-2 protein with greater affinity than the original mouse 4D5 antibody and inhibited the growth of breast cancer cells overexpressing HER-2 (31). Inhibition of growth in vitro was associated with down-modulation of HER-2, inhibition of cell cycle progression as a result of p27 induction, inhibition of angiogenesis, and induction of immune response (32).

In a multicenter phase II clinical trial ($n = 222$), 15% of patients with metastatic breast cancer that had relapsed after chemotherapy responded to trastuzumab used as a single agent (33). More recently, a phase III trial was performed comparing chemotherapy in combination with trastuzumab to chemotherapy alone as first-line therapy in 469 patients with metastatic breast cancer (34). All patients enrolled in this trial overexpressed HER-2 as determined by immunohistochemistry. At a median of 30 months of follow-up, the time to progression for patients receiving both trastuzumab and chemotherapy was 7.4 months compared with 4.6 months for those who received chemotherapy alone. The overall response rate and response duration were also significantly increased in patients who received the combined therapy.

USE OF HER-2 FOR PREDICTING RESPONSE TO TRASTUZUMAB IN BREAST CANCER

On the basis of cell culture and animal model experiments, it is generally believed and highly likely that overexpression of HER-2 is necessary for trastuzumab to induce tumor regression. Consequently, at this stage, trastuzumab should be given only to breast cancer patients showing gene amplification or overexpression of HER-2. Thus, the main clinical use, and the only mandatory use of HER-2 assays at present, is for selecting breast cancer patients with advanced disease for treatment with

trastuzumab. In 2000, an ASCO Expert Panel stated that "unless it can be shown by future work that Herceptin is of benefit in HER-2-normal tumors, use of this antibody will be confined to those patients that have either amplification or overexpression of HER-2" (22).

Although measurement of HER-2 is mandatory before the administration of trastuzumab, controversy exists regarding the optimum type of assay for this marker. Currently, two main types of assay exist, i.e., immunohistochemistry and fluorescent in situ hybridization [FISH; for a review, see Ref. (30)]. Each of these methods has distinct advantages and disadvantages. The advantages of immunohistochemistry include its wide availability, simplicity, and relatively low costs. Its disadvantages include subjectivity in evaluating the staining score, possible loss of HER-2 protein as a result of tissue storage and fixation, and variable results depending on both the antibody and staining procedure used.

In contrast to immunohistochemistry, FISH provides a more objective scoring system. It also has the advantage of a built-in internal control consisting of two HER-2 gene copies in the nonmalignant cells within the specimen. The disadvantages of FISH include its high costs, the requirement for a fluorescence microscope, and inability to preserve the slide for storage and review. Emerging results, however, suggest that FISH is more accurate than immunohistochemistry in predicting both patient outcome and response to trastuzumab (30).

USE OF HER-2 FOR PREDICTING RESPONSE TO HORMONE THERAPY IN BREAST CANCER

At least 20 different studies have investigated the relationship between HER-2 and response to endocrine therapy in patients with breast cancer [for reviews, see Refs. (30, 35, 36)]. For patients with both early and advanced disease, the authors of the majority of these studies concluded that overexpression of HER-2 correlates with either relative resistance or adverse outcome after treatment with hormonal therapy (35, 36).

The studies published to date, however, have the following limitations (36):

- The HER-2 assay was usually performed retrospectively and only in a subset of the patients participating in the relevant clinical trial. This practice could have produced a biased outcome.
- In most of the adjuvant trials, randomly selected untreated controls were not included. These studies may therefore have assessed the prognostic value of HER-2 in patients treated with hormone therapy rather than its predictive value.
- Different types of HER-2 assays as well as different cutoff points were used in the various studies.
- Different forms of hormone therapy were used, and different subgroups of patients were studied in the various trials.

- Most studies contained relatively small numbers of patients and thus were underpowered to show a possible significant predictive effect.
- Most studies used immunohistochemistry to determine HER-2 status. As discussed below, immunohistochemistry has several disadvantages when used for detecting HER-2.

Because of these limitations, the available data are not sufficiently strong to recommend routine use of HER-2 for determining breast cancers likely to be resistant to endocrine therapy. In particular, the value of HER-2 in selecting for hormone resistance has not been validated in a level I evidence study, i.e., in either a large randomized trial or metaanalysis of small-scale prospective or retrospective studies (37). Consequently, the recent ASCO guidelines on breast cancer markers stated that "the use of HER-2 to decide whether to prescribe endocrine therapy either in the adjuvant or metastatic setting is not recommended" (22).

USE OF HER-2 IN PREDICTING RESPONSE TO CHEMOTHERAPY IN BREAST CANCER

The relationship between HER-2 concentrations and response to chemotherapy in breast cancers appears to depend on the type of drug(s) administered. With adjuvant cyclophosphamide, methotrexate, and 5-fluorouracil (CMF), the majority of studies showed a diminished benefit in HER-2-positive compared with HER-2-negative patients [for reviews, see Refs. (30, 35, 36)]. However, it should be stated that patients with cancers overexpressing HER-2 are likely to derive benefit from treatment with CMF-based regimens compared with no treatment. CMF-based therapy should therefore not be withheld from women whose tumors express high amounts of HER-2 and for whom anthracyclines are contraindicated (36).

Because most of the studies relating HER-2 to CMF response suffered from limitations similar to those described above for response to hormonal therapy, assay of HER-2 cannot be recommended at this stage for indicating likely resistance to CMF therapy (22).

Although most published studies suggest that HER-2 overexpression correlates with relative resistance to CMF, increased concentrations may predict enhanced sensitivity to anthracycline-based regimens in the adjuvant setting (30, 35, 36). Thus, the available evidence suggests that patients with HER-2-positive cancers are more likely to respond to anthracycline-based regimens than HER-2-negative patients and that HER-2-positive patients are more likely to benefit from anthracycline-based than alkylating agent-based therapy (30, 35, 36). According to the ASCO statement, "HER-2 may identify patients who particularly benefit from anthracycline-based adjuvant therapy, but levels of HER-2 should not be used to exclude patients from this type of treatment" (22).

p53

CHEMISTRY AND BIOLOGY

The p53 tumor suppressor gene is located on chromosome 17p and encodes a 393-amino acid nuclear phosphoprotein with a molecular mass of 53 kDa [for a review, see Ref. (38)]. Functionally, p53 acts as a transcriptional factor and like all known transcriptional factors contains several distinct domains (38). The amino-terminal region is thought to be involved in transactivation, whereas the central domain mediates sequence-specific DNA binding. The carboxy terminus is responsible for oligomerization, p53 being functionally active as a tetramer (38).

p53 is the most commonly mutated gene in human cancers (38). Most of the mutations are of the missense type and occur in the DNA-binding domain. The consequence of many of these mutations is loss of the ability of p53 to bind to DNA in a sequence-specific manner.

p53 controls the expression of multiple genes that are broadly divided into four categories, i.e., cell cycle inhibition, promotion of apoptosis, control of genome stability, and inhibition of angiogenesis (39). Being involved in such a variety of critical cellular activities, it is not surprising that loss of p53 function is so damaging and that such losses occur in almost all human cancers.

USE OF p53 FOR PREDICTING RESPONSE TO CHEMOTHERAPY

As mentioned above, one of the established functions of p53 is induction of apoptosis. It is now widely believed that many anticancer agents induce tumor regression, at least in part, by causing apoptosis (40). Thus, disruption of the apoptotic process, e.g., by loss or mutation in the p53 gene, might therefore be expected to reduce response to treatment or cause drug resistance.

Evidence for a link between dysfunctional p53 and failure to respond to therapy has been found in several model systems (41). For example, p53-null mice have been found to be resistant to apoptosis induced by 5-fluorouracil (5-FU) in cancers of the small intestine, to arabinofuranosyl in cancers of sympathetic neurons, and to Adriamycin in cancers of the thymus, spleen, and small intestine. Furthermore, reintroduction of wild-type p53 into mutant cell lines and xenographs led to induction of apoptosis and tumor regression (41).

The relationship between p53 status and response to therapy in human cancers is less clear. Elledge and Allred (42) reviewed the literature on the relationship between alterations in p53 and response to different therapies in patients with breast cancer. Of 17 studies identified, 9 found no correlation between abnormalities in p53 and response, 5 showed that altered p53 predicted resistance, and 3 concluded that dysfunctional p53 was related to sensitivity. Similarly, in other cancers, conflicting findings exist on the relationship between p53 and response to chemotherapy (43). Possible reasons for the conflicting data have been discussed previously (44) and include:

- The optimum type of assay for assessing p53 status is unknown, i.e., whether to analyze for gene mutation, protein production, or use a functional assay;
- As stated above, p53 protein has multiple activities. Its capacity to induce apoptosis may depend on criteria such as type of drug, drug dose, tumor type, and mutation spectrum of the tumor; and
- Apoptotic pathways unrelated to p53 may be important in inducing cell death in some tumors.

Clearly, at present p53 cannot be used to select for either sensitivity or resistance to anticancer treatments. Similarly, other proteins involved in apoptosis, such as bcl-2, bax, CD95, or specific caspases, cannot currently be used for determining sensitivity or resistance to anticancer treatments [for a review, see Ref. (45)].

ATP-Dependent Transporters

CHEMISTRY AND BIOLOGY

Cell lines grown in the presence of a single cytotoxic agent frequently become cross-resistant to many functionally and structurally unrelated agents. The best known molecular mechanism responsible for this type of multidrug resistance is overproduction of membrane proteins known as ATP-dependent efflux pumps (46, 47). These proteins are characterized by an ATP-binding cassette or domain and are thus known as the ABC superfamily of transporters or ATP-dependent transporters. To date, 48 human ABC genes have been identified and divided into seven distinct subfamilies based on their sequence homology and domain structure [for a review, see Ref. (46)].

The prototype member, ABCB1 (also known as P-glycoprotein, P-170, PGP, or MDR1) is a broad-spectrum multidrug efflux pump that possess 12 transmembrane domains and 2 ATP-binding sites (46, 47). Physiologically, ABCB1 is thought to play a role in extruding neutral and cationic toxins out of cells. Anticancer drugs shown to be substrates for ABCB1 include anthracyclines (e.g., doxorubicin), vinca alkaloids (e.g., vincristine), epipodophylotoxins (e.g., etoposide), and taxanes (e.g., paclitaxel and docetaxol) (47).

Another widely studied transporter is ABCC1, which is also known as MRP-1. Structurally, MRP-1 is similar to P-glycoprotein except for an amino-terminal extension that contains 5 transmembrane domains, giving a total of 17 transmembrane sequences (46). MRP-1 has been found to extrude glutathione-conjugated derivatives of multiple toxic compounds as well as organic ions from cells. Cytotoxic drugs that are substrates for MRP-1 include doxorubicin, methotrexate, etoposide, and vincristine (46).

A non-ABC transporter was recently shown to confer multiple drug resistance in lung cancer cells and was given the name lung cancer resistance-related protein (48). Lung cancer resistance-related protein is a vault protein and, in contrast to the ABC transporters, does not possess an ATP-binding domain. Rather, vault proteins

are large ribonucleoprotein complexes with a hollow barrel-shaped structure. These complexes are thought to compartmentalize drugs away from their intracellular targets and extrude these molecules by a vesicle-mediated exocytosis efflux mechanism.

USE OF ATP-DEPENDENT DRUG TRANSPORTERS FOR PREDICTING RESPONSE TO CHEMOTHERAPY

Multiple small-scale retrospective studies have evaluated the relationship between concentrations of specific drug transporters (especially p170) and response to different chemotherapeutic regimes in a variety of malignancies [for reviews, see Refs. (46, 47, 49)]. In 1997, Trock et al. (50) performed a metaanalysis of 31 published studies on the relationship between p170 and chemotherapy resistance in breast cancer. In total, 31 studies were identified and evaluated. Overall, 42% of the tumors overexpressed p170 mRNA or protein, although there was wide variation in the percentage positivity in the different reports. p170 concentrations increased after therapy, and this increase was associated with lack of response to treatment (50). Five studies with a total of 115 participants assayed p170 before treatment. Although there was a trend, the relationship between pretreatment concentrations of p170 and response to therapy in this subgroup was not significant ($P = 0.088$).

Compared with breast cancer, less work has been performed on p170 in other human cancers. Some, but not all, investigators have found a correlation between p170 expression and treatment outcomes in acute myeloid leukemia [for a review, see Ref. (51)]. In osteosarcoma, a recent prospective multicenter study found no relationship between p170 expression and response to neoadjuvant chemotherapy (52). Clearly, assay of p170, or indeed any of the other ATP transporters mentioned above, cannot be used at present for predicting clinical resistance.

Thymidylate Synthase

CHEMISTRY AND BIOLOGY

Thymidylate synthase (TS) is a 36-kDa dimeric protein that catalyzes methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), using the reduced folate 5,10-methylenetetrahydrofolate as the methyl donor (53, 54). This reaction provides the only de novo source of thymidylate which is essential for DNA synthesis.

TS is a target for several chemotherapeutic agents, including the fluoropyrimidines, 5-FU and 5-fluorodeoxyuridine, and the antifolate, tomudex. 5-FU, in particular, is used to treat several different malignancies, such as those of the gastrointestinal tract, head and neck, and breast. In colorectal cancer, 5-FU-based therapy has been found to increase both disease-free and overall survival in patients with resected stage 3 disease (54). In advanced colorectal cancer, however, response rates are only ~20% (4, 54).

To inhibit TS, 5-FU is first converted to 5-fluorode-

oxyuridine monophosphate, which forms a covalent complex with TS in the presence of 5,10-methylenetetrahydrofolate (53, 54). Inhibition of TS leads to depletion of initially dTMP and later of dTTP and to an accumulation of dUMP. As a consequence, dUTP is incorporated into DNA because of lack of the natural substrate, dTTP. Its subsequent excision leads to DNA damage and apoptosis [for a review, see Ref. (54)]. A different 5-FU metabolite, fluorouridine monophosphate, is incorporated into RNA, disrupting normal RNA processing and function.

USE OF TS IN PREDICTING RESPONSE TO 5-FU IN COLORECTAL CANCER

Studies using colorectal cancer cell lines initially suggested an association between TS concentrations and response to 5-FU (55). It was later shown that transfection of colonic cancer cells with TS cDNA led to resistance to 5-FU (56). Consistent with these results, several preliminary studies in patients with advanced colorectal cancer have shown that high concentrations of TS correlate with resistance to 5-FU-based chemotherapy, i.e., patients with high tumor concentrations of TS rarely respond to infusion treatment with 5-FU, whereas patients with low concentrations display response rates higher than expected [for reviews, see Refs. (54, 57)].

Recently, Popat et al. (58) carried out a systematic review and metaanalysis of published studies relating TS concentrations to outcome in patients with advanced colorectal cancer treated with diverse TS inhibitors. In total, 13 studies containing 887 patients were identified. Of these, 12 were deemed to be suitable for pooling of the overall survival data. Following a pooled analysis, the overall hazard ratio associated with high concentrations of TS for overall survival was 1.74 (95% confidence interval, 1.34–2.26). The impact of TS concentrations on outcome, however, was dependent on whether the TS assay was carried out on the primary tumor or on a metastatic lesion. For example, if TS concentrations were determined on the metastatic lesion, the hazard ratio was 2.39 (95% confidence interval, 1.43–4.01). On the other hand, if TS was measured on the primary tumor, the hazard ratio was only 1.33 (95% confidence interval, 1.07–1.61). It thus appears that for predicting outcome in patients with advanced colorectal cancer treated with TS inhibitors that TS concentrations must be measured on the metastatic lesion.

Other Individual Predictive Markers

Other potential predictive markers for anticancer therapies are listed in Table 3. It is important to point out that none of the markers included in Table 3 have yet been validated for clinical use.

Microarray

A microarray consists of multiple rows of oligonucleotides or cDNAs lined up in an orderly manner on a small glass or silica slide (usually only 1–2 cm square). With

Table 3. Potential markers for predicting response or resistance to specific cancer therapies.

Marker	Malignancy	Drug(s)	Ref(s).
MGMT ^a	Glioma	Alkylating agents	(68)
Topoisomerase II α	Breast	Anthracyclines	(69)
uPA and PAI-1	Breast	Chemotherapy (adjuvant)	(70, 71)
uPA and PAI-1	Breast	Hormone (advanced disease)	(72, 73)
EGFR	Breast	Hormone (advanced disease)	(74)
pS2	Breast	Hormone (early disease)	(75)
VEGF	Breast	Hormone and chemotherapy (advanced disease)	(76)
VEGF	Breast	Radiotherapy	(77)
TIMP-3	Breast	Hormone therapy	(78)
YB-1	Breast	Chemotherapy	(79)
c-kit mutations	GIST	Imatinib	(80)
EGFR mutations	NSCL cancer	Gefitinib	(81, 82)

^a MGMT, methylguanine methyl transferase; uPA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor 1; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; GIST, gastrointestinal stromal tumor; NSCL, non-small cell lung.

microarrays, the expression of tens of thousands of genes in a biological sample can be measured simultaneously [for a review, see Ref. (59)]. As pointed out by Winegard (60), the use of microarrays for predicting patient outcome has two major advantages compared with the use of single markers: (a) microarrays permit the screening of multiple genes without previous knowledge of which genes might be predictive; and (b) with microarrays, groups of genes rather than single genes, when investigated together, may be a more reliable indicator of clinical response.

Early studies on the use of microarrays for predicting anticancer drug response focused on cell lines (61, 62). These studies showed that, at least for some of the compounds, the gene expression profile of untreated cells was capable of being used for chemosensitivity testing (63). To date, only a few preliminary studies have been published on the use of microarrays for predicting clinical response or resistance to anticancer agents.

In a phase II trial on 24 patients with locally advanced breast cancer, Chang et al. (63) found that 92 genes were differentially expressed in tumors from patients that were sensitive or resistant to neoadjuvant (i.e., given before surgery) docetaxol therapy. Sensitivity or resistance was defined on the basis of residual tumor at the end of treatment. Using this gene signature, the authors could correctly classify 10 of 11 sensitive tumors and 11 of 13 resistant tumors. The results were subsequently validated in an independent set of only six patients. Sensitive tumors displayed increased expression of genes involved in the cell cycle, cytoskeleton, adhesion, protein transport, and apoptosis, whereas resistant tumors had increased expression of transcription and signal transduction genes.

Ayers et al. (64) also used microarrays in an attempt to identify genes predictive of response to neoadjuvant therapy in patients with breast cancer. In this study, the chemotherapy used was sequential paclitaxel and 5-FU + doxorubicin + cyclophosphamide, the number of patients investigated was 42 (24 used for discovery and 18 for

independent validation), and the endpoint was pathologic complete response. Using a 74-gene signature, the authors obtained a 78% (14 of 18) predictive accuracy in the validation group.

Another malignancy for which microarrays have been used to identify therapy-predictive markers is acute lymphoblastic leukemia (ALL). Approximately 80% of children with childhood ALL are cured by chemotherapy. In an attempt to address the mechanisms of resistance, Holleman et al. (65) investigated ALL cells from 173 children for in vitro sensitivity to daunorubicin, vincristine, prednisolone, or asparaginase. They then used gene expression profiling with 14 500 probe sets to select differentially expressed genes in drug-sensitive and -insensitive ALL cells.

Overall, 172 gene probe sets were found to be differentially expressed in sensitive and resistant B-lineage leukemic cells. These included 22 gene probes for daunorubicin, 59 for vincristine, 42 for prednisolone, and 54 for asparaginase. Overall, the probes correctly assigned the drug sensitivity status of 86 of 105 cases for daunorubicin, 84 of 104 for vincristine, 66 of 75 cases with respect to prednisolone, and 83 of 106 cases with respect to asparaginase.

Combined gene expression for resistance to the four agents was associated with a significantly increased probability of disease relapse. The combined resistance score was also predictive of treatment outcome in a multivariate model that included age of patient, ALL genetic subtype, ALL lineage, and leukocyte number at diagnosis. These results were confirmed in an independent population of patients treated similarly to that in the original 173 patients (65).

Hofmann et al. (66) used microarrays to identify genes conferring resistance to the tyrosine kinase inhibitor imatinib (Glivec) in patients with ALL. This study was carried out on 19 adult patients with Philadelphia chromosome-positive ALL who were enrolled in a phase II trial investigating the safety and efficacy of imatinib. Using 95

genes, the authors were able to separate all of the imatinib-sensitive from the imatinib-resistant cases. Among the genes highly expressed in the resistant ALL cells were Bruton's tyrosine kinase and two ATP synthetases (ATP5A1 and ATP5C1). Genes with decreased expression in the cells included the proapoptotic gene BAK1 and the cell cycle control gene p15INK4B.

Conclusions

The prototype predictive markers in oncology are the ER and PR. These markers were initially introduced ~30 years ago to predict response to hormone therapy in patients with advanced breast cancer. Today, their principal application is selecting patients with early breast cancer likely to respond to hormone therapy. A more recently introduced predictive marker is HER-2, which is used for selecting patients with metastatic breast cancer for treatment with trastuzumab. Further work, including validation in level 1 evidence studies, is necessary before HER-2 can be used for predicting response to either chemotherapy or hormone therapy in patients with breast cancer. Further research will also be necessary to establish whether molecules involved in apoptosis or drug efflux mechanisms are associated with clinical response. However, because drug resistance or response almost certainly depends on the interplay of multiple genes, it is likely that multiple markers will have to be assessed to have reliable predictive tests (67). The most convenient ways of simultaneously determining such multiple markers is likely to be customized DNA microarrays or proteomics.

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