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Review

Exploiting the hallmarks of cancer: the future conquest of breast cancer☆

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Abstract

What separates a malignant from a normal cell? This question has occupied scientists for decades. Although a simple answer remains elusive, several hallmarks of malignancy have been identified. These critical features include uncontrolled proliferation, insensitivity to negative growth regulation, evasion of apoptosis, lack of senescence, invasion and metastasis, angiogenesis and genomic elasticity. Existing therapies predominantly target proliferation either with cytotoxic agents, ionising radiation or more targeted attacks on growth factor signalling pathways. Our most successful therapies to date inhibit proliferation via the oestrogen receptor (ER) and HER2 pathways. Further improvements in therapy must attack the other hallmarks of malignancy and will undoubtedly be accompanied by a better means of individual patient selection for such therapies. Indeed, each of these hallmarks presents a therapeutic opportunity. To believe otherwise would be to assume that a feature is both biologically crucial, yet therapeutically unimportant, an unlikely paradox. Here, we suggest the hallmarks of malignancy as a conceptual framework for understanding novel breast cancer therapies.

Keywords: Breast cancer; Hallmarks of cancer; Targeted therapies; Biology

1. Introduction

Now mark me how I am undone.
William Shakespeare, *Richard II*

Oncologists typically thought of systemic therapies for breast cancer as belonging to one of two categories: either cytotoxic chemotherapy or hormonal therapy. Although this simple approach served us well for decades, the recent development of trastuzumab showed its inadequacies. Trastuzumab, neither broadly cytotoxic nor a classic hormonal manipulation, did not fit. A third category, biological therapy (alternatively, targeted therapy), has entered the lexicon, but is vague and unsatisfying. Isn't hormonal therapy biological? Isn't 5-fluorouracil targeted?

The growing complexity of cancer treatment, and more particularly of the biological basis underlying these therapies, suggests we re-consider our conceptual approach to the treatment of breast, and indeed all, cancer. Hanahan and Weinberg, in discussing "The Hallmarks of Cancer", provide a framework for approaching these novel therapies (Table 1) [1]. This landmark review identified, in broad terms, seven critical features—the cancer cell's 'tool kit'—responsible for the phenotype we recognise as cancer. We argue in this review that novel therapies for breast cancer can and should be viewed within the framework of these hallmarks of cancer.

2. Self-sufficiency in growth signals

During normal growth and differentiation, and in the subsequent life of the host, cell proliferation is rigidly controlled. Cancer cells escape the normal growth control and proliferate unchecked. This escape may occur via mutations that result in overexpression of differentially regulated growth factors or their receptors. Tar-

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geting self-sufficiency in growth signals, the oldest 'new' idea in breast cancer treatment, has had profound effects. Initially proposed as adjunctive therapy by Schinzinger in 1889, Beatson introduced ovariectomy into clinical practice in 1896 [2]. The biological underpinnings for hormonal therapy remained unexplained until the identification of the oestrogen receptor (ER) in the 1960s [3]. The ER remains arguably the most important growth factor receptor identified for breast cancer. Adjuvant hormonal therapies, whether by ovarian ablation or with tamoxifen, have a bigger impact on recurrence and survival than any other treatment.

ER targeting began with empirical observation, followed half a century later by identification of the target. In contrast, Slamon and colleagues reversed this process, starting with the biological importance of HER2 and then designing a HER2-specific therapy. HER2 is overexpressed in 20–25% of breast cancers and associated with a poor prognosis [4]. The addition of trastuzumab, a monoclonal antibody recognising HER2, to standard chemotherapy resulted in higher response rates (50% versus 32%, $P<0.001$) and prolonged survival (median, 25.1 versus 20.3 months, $P=0.046$) in patients with metastatic breast cancer [5]. Adjuvant trials of trastuzumab are currently ongoing worldwide.

Can we target other aspects of growth self-sufficiency in breast cancer? Leaving aside the introduction of new agents for old targets (e.g. the introduction of third-generation aromatase inhibitors in both the metastatic and adjuvant settings) [6], an increased understanding of signalling pathways and the identification of other growth factors point to novel opportunities for therapy.

First, it now seems clear that there is considerable cross-talk between the HER2 and ER signalling pathways [7]. HER2-positive breast cancers are generally less sensitive to ER modulation (particularly with tamoxifen) [8,9]. This insensitivity may reflect HER2′s regulation of ER coactivators such as AIB1, mediated via the MAP kinase pathway and p27 [10,11]. Recent preclinical work suggests that interruption of HER signalling pathways (whether via HER1 [12] or HER2 [13]) reverses resistance to tamoxifen. This cross-talk provides the rationale for clinical trials combining ER- and HER (1 or 2)-targeted therapies.

In addition, while the first generation of targeting involved receptors such as HER2, current efforts are underway to target downstream mediators of cell surface receptors (e.g. ras, MAP kinase and PI3 kinase pathways). Several agents targeting such downstream mediators are in clinical development. Farnesyl transferase-targeting agents have already shown clinical activity in breast cancer [14].

Growth factors such as the transforming growth factor alpha (TGF-α) and insulin-like growth factor-1 (IGF-1) stimulate benign breast growth and may be important drivers of self-sufficient growth in breast cancers. TGF-α is a principal growth factor in the normal and neoplastic development of the mammary gland [15]. It binds the epidermal growth factor receptor (EGFR, HER1) and stimulates epithelial growth in the virgin and pregnant mouse mammary gland. TGF-α expression can be detected in breast cancer cells *in vivo* and *in vitro*; overexpression of TGF-α elicits partial transformation or immortalized human and rodent mammary epithelial cells [16]. Intact EGFR is present in approximately 40% of breast cancers with even higher levels of expression if the truncated EGFRvIII form that lacks an extracellular domain is included [17,18].

Table 1
The hallmarks of cancer[a]

1. Self-sufficiency in growth signals
2. Insensitivity to antigrowth signals
3. Evading apoptosis
4. Limitless replicative potential
5. Sustained angiogenesis
6. Tissue invasion and metastasis
7. Genomic instability

[a] From Hanahan and Weinberg [1].

Initial clinical trials of EGFR-blocking agents have been presented recently for both gefitinib and erlotinib [19,20]. These phase II trials, conducted in heavily pretreated patients, have proven disappointing with few responding patients. Whether this reflects the disease setting (advanced-stage, highly refractory disease), lack of patient selection or inability of EGFR inhibition alone to limit self-sufficient growth is unknown. The successes of tamoxifen and trastuzumab were not realised until patients were selected for expression of ER or HER2; both would be discarded as drugs with minimal activity if they were tested in patients with ER- or HER2-negative tumours. We currently lack sufficient knowledge and validated assays to apply EGFR inhibitors as 'targeted therapy' in any meaningful sense.

IGF-1 signalling is required for cell cycle progression [21] and appears to be a prerequisite for malignant transformation [22]. IGF-1 receptor activation is associated with the growth of hormone-dependent breast cancers; the importance of IGF-1 in ER-negative tumours is less clear [23]. The synergistic activity of ER and IGF-1 receptor signalling provides the rationale for combined treatment with anti-oestrogens and IGF-1 inhibitors. Initial trials of somatostatin analogues (that decrease hepatic IGF-1 synthesis) in patients with metastatic disease were disappointing [24–26]; adjuvant trials were limited by toxicity [27]. Preclinical development of more specific IGF-1 or IGF-1 receptor inhibitors continues.

3. Insensitivity to antigrowth signals

Growth and development constitute an intricate network of positive and negative feedback loops. In human cancer, not only are the positive stimulators overactive; the negative controls fail to function properly. Many of the negative feedback loops modulate cyclin function to control cell-cycle progression. Altered expression of the cyclins, or the cyclin-dependent kinases (cdks) and cdk inhibitors that regulate cyclin function is frequently found in malignancy.

Cyclin D1 is expressed relatively early in breast carcinogenesis, commonly found in atypical hyperplasias and ductal carcinoma *in situ* [28]. Overexpression of cyclins (e.g. Cyclin E) and related cdks is associated with impaired outcome in breast cancer [29]. Similarly, lack of expression of cdk inhibitors (p21, p27waf) portends an impaired outcome [30–32]. Together these data suggest the importance of insensitivity to inhibitory signals in breast cancer.

Pharmacological cdk inhibitors are currently in development, at both the laboratory and clinical level. The first of these agents to enter clinical trials is flavopiridol, a derivative of the Indian plant, *Dysoxylun binectariferum*. Flavopiridol blocks cell cycle progression through its actions on Cyclins D1, D2, D3 and Cyclin E [33]. In phase I trials, gastrointestinal toxicity was dose-limiting with prolonged infusions, while myelosuppression was most common with daily therapy. Symptomatic orthostatic hypotension was also frequently noted [34,35]. Other agents with greater specificity are currently in development.

In addition to specific cdk inhibitors in development, several other agents inhibit cdks. Anti-oestrogen therapy decreases Cyclin D, cdk4 and cdk2 expression and increases p21 (waf/cip1) expression. In this setting, p21 negatively regulates cdk4 expression [36,37]. Similarly, treatment with AG-1478, an EGFR inhibitor, in mouse mammary tumour virus (MMTV)/Neu + MMTV/transforming growth factor alpha bigenic mice decreases cdk2, MAPK activation and Cyclin D1 and increases the expression of cdk inhibitor p27 (Kip1)[38]. Other agents shown to negatively regulate cyclin or cdks include the retinoids, paclitaxel, trastuzumab and histone deacetylase (HDAC) inhibitors [33,39,40].

4. Evasion of apoptosis

Cancer cells face many challenges to their survival and, under normal (physiological) circumstances, these challenges would lead to cell death through activation of apoptotic pathways. Apoptosis (programmed cell death) may be triggered through either an extrinsic or intrinsic pathway. The extrinsic pathway is mediated through transmembrane death receptors (e.g. TNF receptor, TNF-α Related Apoptosis Inducing Ligand (TRAIL) receptor, or CD95), which activate activator caspases (caspases 8 and 10) leading to downstream activation of effector caspases. The intrinsic pathway involves mitochondrial membrane disruption, and is regulated by pro- and anti-apoptotic molecules such as Bak and Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic). The p53 protein, the so-called 'guardian of the genome', can induce apoptosis via both intrinsic and extrinsic pathways.

Unsurprisingly, genetic alterations in apoptotic pathway members are a common feature of tumour progression. Mutated p53 is common in many solid tumours, including breast cancers, as are alterations in several pro- and anti-apoptotic genes and the genes that in turn regulate them. Upregulation of the anti-apoptotic pathways produces broad resistance to chemotherapeutic agents and ionising radiation [41,42].

Numerous therapeutic approaches to reversing apoptosis evasion are currently under development. These include gene therapy strategies aimed at replenishing wild-type p53, agents targeting crucial intrinsic pathway mediators such as Bcl-2 [43], and agents that represent analogues of death signals such as TRAIL [44]. Several of these approaches are poised to enter clinical trials in breast cancers. Such agents may magnify chemotherapeutic effects.

5. Limitless replicative potential

Unlike Ponce de Leon, cancers have found the fountain of youth. In normal cells senescence is associated with the progressive loss of telomeres, the repetitive DNA sequences capping each chromosome. With each replicative cycle, telomeres become progressively shorter, eventually resulting in senescence and cell death. Tumours commonly express the enzyme telomerase, which protects the telomeres from shortening. In essence, tumours never grow old. Expression of hTERT, the human telomerase catalytic subunit gene, is common in breast cancers [45], including pre-invasive tumours [46,47]. Telomerase expression is associated with lymphovascular invasion, nodal metastasis [48] and a higher relapse rate following initial therapy [49]. Several factors affect telomerase expression in breast cancers. Oestrogen [50] and progesterone [51] stimulate, while tamoxifen [52] and wild-type p53 [47,53] inhibit telomerase activity.

Can we target telomerase with therapeutic intent? This is unknown at present. Inhibiting telomerase would not lead to immediate tumour cell senescence and death. However, inhibition of telomerase in human breast cancer cells renders them more sensitive to topoisomerase inhibition [54], suggesting the potential for combinatorial activity with standard agents such as

doxorubicin. Specific telomerase-inhibiting agents are currently in development [52,55], and should enter the clinic in the near future.

6. Sustained angiogenesis

Invasion and metastasis of breast cancers, indeed of all solid tumours, depends on angiogenesis, the formation of new blood vessels to nourish the tumour [56]. The normal vasculature is quiescent in healthy adults, with each endothelial cell dividing once every 10 years; active angiogenesis is required only for wound healing, endometrial proliferation, postlactational mammary gland involution and pregnancy. In contrast, tissue remodelling and angiogenesis are crucial for the growth and metastasis of breast cancers and provides an attractive therapeutic target that may have limited (at least theoretically) toxicity.

Our burgeoning understanding of angiogenesis has fostered the development of agents targeting specific steps in the angiogenic cascade, many of which have entered the clinic. A detailed list of agents in clinical development can be obtained from the Angiogenesis Foundation (http://www.angiogenesis.org) or from the National Cancer Institute (http://cancernet.nci.nih.gov). While the number of ongoing phase I and II trials has grown rapidly, few have been reported in the peer-reviewed literature. Only one phase III trial in breast cancer has been completed to date.

Angiogenesis requires stimulation of vascular endothelial cells through the release of angiogenic peptides, of which the vascular endothelial growth factor (VEGF) is the most potent. An antibody directed against VEGF (bevacizumab, Avastin, Genentech) produced an objective response or prolonged stable disease in 17% of patients with previously treated metastatic breast cancer; 4 patients continued therapy without progression for over 12 months [57,58]. In a recently reported phase III trial of 462 patients with anthracycline- and taxane-refractory disease, the addition of bevacizumab to capecitabine significantly increased the response rates (investigator-designated 19.1% versus 30.2%; $P = 0.006$), but did not prolong median progression-free survival (4.17 versus 4.86 months.; hazard ratio = 0.98) [59]. A phase III trial of bevacizumab in patients with newly diagnosed metastatic disease is ongoing (Fig. 1), and several other VEGF-targeting agents are currently in development.

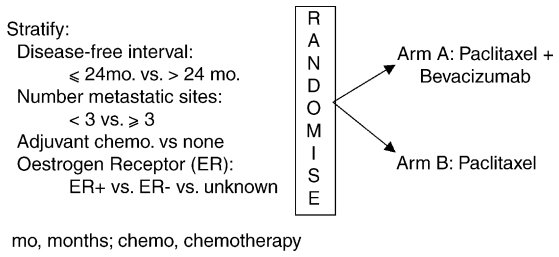

Fig. 1. E2100. Phase III trial for patients with previously untreated metastatic breast cancer. mo, months; chemo, chemotherapy.

7. Tissue invasion and metastasis

Epithelial tumours such as breast cancer frequently derive from normal cells populating the inner lining of ductal structures surrounded by basement membranes. The ability to invade through basement membranes, which characterise the transition from a non-invasive to an invasive cancer, is a hallmark of the malignant phenotype. Metastasis is an extension of local invasion. Following invasion, cancer cells transit the extracellular matrix, intravasate, and traverse blood vessels to lodge at a distant site, extravasate and grow as a metastatic focus.

Tumours secrete proteases such as the matrix metalloproteinases (MMPs) to degrade the basement membrane and surrounding stroma. Overall expression of MMP-2 and MMP-9 has been associated with the grade and stage of breast cancer [60,61] and increased serum levels are found in patients with metastatic disease [62]. Inhibition of the MMPs decreases metastasis and local tumour growth in mouse xenografts [63]. Several broad spectrum MMP inhibitors have entered (and exited) clinical trials when phase III trials failed to find a clinically meaningful improvement in outcome [64–66]. Chronic therapy with the broad spectrum MMP inhibitor, marimastat, failed to delay progression in patients responding or stable after initial chemotherapy for metastatic breast cancer [67]. Although, theoretically, use of the MMP inhibitors in the adjuvant setting would be expected to provide the greatest benefit, musculoskeletal toxicity prevents such use [68].

Tumours may exploit other proteases besides the MMPs. Membrane-associated urokinase-type plasminogen activator (uPA) expression and the ratio of uPA to plasminogen activator inhibitor-1 (PAI-1) are associated with an impaired survival or local relapse [69–72]. uPA inhibitors may have therapeutic potential, but clinical development has thus far been limited by toxicity.

8. Genomic instability

A final part of the cancer 'tool kit' is genomic instability, the progressive destabilisation of the cancer cell's genome. The increasing frequency of mutational events accompanying tumour progression provides a driving force for several of the hallmarks of malignancy (e.g. the tendency of cancers to increase the number of pro-angiogenic and growth proliferation factors over time), as well as the ability of cancers to become increasingly resistant to therapeutic attack.

Genomic instability may be directed related to the cause of the breast cancer (e.g. in the case of BRCA1 and 2) [73,74] or may develop during tumour progression, but in any event is common [75]. Several of the hallmarks of cancer described above are associated with genomic instability. For instance, the ability to evade apoptosis through alterations in p53 [76,77] and Bcl-2 [78], as well as shortened telomere length [79], are all associated with increased genomic instability in breast cancers. Similarly, insensitivity to negative growth signals via overexpression of Cyclin D1 is associated with increased genomic instability [80]. In addition, human breast cancer cells frequently contain an error-prone DNA replication apparatus [81,82].

Can we target genomic instability as a means of decreasing life-threatening developments such as drug resistance? At present, this target seems too general to approach therapeutically; screening and early detection will continue to be an important tool. Genomic instability remains an elusive if important problem barring the conquest of breast cancer.

9. Emerging technology and the hallmarks of cancer

Our ability to apply the hallmarks of cancer to clinical practice has been limited by the insufficiency of both our crude testing methods and our knowledge of breast cancer biology. The evolving genomic and proteomic techniques now allow the interrogation of literally thousands of genes and proteins simultaneously—exponentially increasing our knowledge. These technologies are now rapidly entering the clinic.

The first harvest of these technologies is now coming into focus. Genomic analysis of human breast cancers, using cDNA microarrays (so-called 'gene chips') reveals that what we call breast cancer based on morphology in fact consists of several (perhaps five or six) distinct subtypes [83]. These subtypes (or should we consider them separate cancers?), unsurprisingly have different 'tool kits': for instance, different mechanisms of growth signal sufficiency. Furthermore, cDNA microarray analysis can, with some specificity, distinguish patients at a high and low risk of relapse [84]. Perhaps more importantly, emerging evidence suggests that specific gene expression patterns, measurable by cDNA microarray technology, predict clinically important therapeutic outcomes, such as achieving a pathological complete response with chemotherapy [85].

10. Everything old is new again

These emerging technologies may have remarkable therapeutic consequences. Let us assume that the use of 'gene chip' or 'protein chip' technology becomes widespread in the near future. What might this imply for therapy and drug development? First, we will be able to identify which 'tool kit' a patient's tumour employs. Second, we will treat patients with agents chosen to thwart the specific 'tool kit' employed by their cancers. At least partly individualised therapy in a sense we previously did not dare dream about becomes routine. This consequence seems straightforward enough.

Less straightforward, but equally appealing, is another possibility. Over the past four decades, the drug development community has thrown away literally hundreds of agents. Some of these compounds were discarded because of unacceptable toxicity, some because of insufficient efficacy, and some failed on both fronts. A relatively inactive agent in an unselected population of breast cancer patients may be a highly active agent in a tightly defined subpopulation (if we just knew the proper definition). One has only to look at the history of trastuzumab—active in HER-2-positive patients, inactive in HER-2-negative—to realise that we may have tossed away numerous potentially useful agents over the years through inadequate targeting.

In the era where genomics, proteomics and the hallmarks of cancer converge, might we not be able to resurrect previous developmental failures? Certainly it makes as much sense (economically and ethically) to re-explore an agent that has already been tested in the preclinical, phase I and phase II settings as it does to develop an entirely new agent—assuming, of course, that the agent can be shown to target a highly specific, readily identifiable subpopulation with a specific 'tool kit'. In this sense, 'everything old is new again': we may be able to utilise the new understanding of breast cancer biology to reinvigorate many an old agent.

11. Conclusions

In the course of this review, we have suggested that clinicians and researchers begin to think of therapies for breast cancer—and, indeed, for all cancers—in a new way. This approach specifically emphasises the biological basis of the disease as a roadmap for treatment and novel agent development. While the components of the cancer 'tool kit' may vary from patient to patient, the variations are not infinite, and the hallmarks required by 'successful' cancers are, in fact, remarkably consistent.

An approach centred on the hallmarks of cancer should have many advantages. Because the 'tool kit' is a finite package, and because cancer cells rely heavily on this limited repertoire for their survival, cancer should ultimately represent a *calculable* problem. Emerging technologies will allow a thorough and detailed interrogation of the malignant genotype and phenotype of individual cancers. This interrogation will in turn lead

to a successful therapeutic prosecution. In effect, breast cancer should become just another disease, not some frightening and mysterious event. Its strengths will become weaknesses.

We have accomplished a great deal despite the fact that our current therapies predominantly target (although few are actually 'targeted') just one of these hallmarks. The personal and public health benefits to be gained by exploiting other components of the cancer 'tool kit' are sure to be equally profound. In the words of the English novelist Eden Phillpotts, "The universe is full of magical things patiently waiting for our wits to grow sharper."

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