disease over 10 years [21], a clean colon at this age might mean that no further follow-up is needed. For high-risk group screening, the most pressing need is for methods to accurately quantify an individual's risk of subsequent cancer. Genetic markers may be useful in this respect. Screening may then be tailored according to individual risk either by changing the age at which screening starts, the frequency of testing or finally by employing more sensitive tests, either alone or in combination. The converse of this may be the identification of a population subset with a particularly low risk of the disease who do not need to be screened at all.

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Cathepsin D in Breast Cancer: A Tissue Marker Associated with Metastasis

BREAST CANCER kills via its metastatic potential and removal of a primary tumour is not always sufficient to cure a patient who may develop distant metastasis. Tumour size and node invasiveness are the most potent classical prognostic markers for predicting tumour aggressiveness. However, in 20–30% of node-negative patients, breast cancer will relapse and new predictive markers are required to help in making treatment decisions[1].

10 YEARS OF RESEARCH ON CATHEPSIN D IN ONCOLOGY

The first biologically active molecular markers used were oestrogen receptors (ER) and progesterone receptors which are now routinely assayed in the cytosol of primary tumours. Since about 30% of oestrogen receptor-positive tumours are unresponsive to hormone therapy, our laboratory searched for better hormone responsiveness markers and found a 52 kD protein secreted by metastatic breast cancer cell lines and

Editorial dedicated to the memory of William McGuire (San Antonio), a pioneer in the field of prognostic markers in breast cancer, who died on 25 March 1992.
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Table 1. Prognostic value of cathepsin D level in breast cancer cytosol (multivariate analysis)

References	Median follow-up (month)	Number of patients	Group	Cut-off (pmol/mg protein)	*P* value (Cox) Relapse-free survival	*P* value (Cox) Overall survival
1. Thorpe *et al.* (Copenhagen) *Cancer Res* 1989, **49**, 6008.	72	396	Pre-men.	78	0.06	0.3
			Post-men.	24	0.039	0.089
2. Spyratos *et al.* (St Cloud) *The Lancet* 1989, ii, 1115.	54	120	Total	45/70	<0.001/<0.001	NS/0.04
			N-	45/70	<0.01/0.001	
3. Tandon *et al.* (San Antonio) *N Engl J Med* 1990, **322**, 297.	84	188	N-	75*	<0.0001	<0.0001
4. Romain *et al.* (Marseille) *Bull Cancer* 1990, **77**, 439.	58	85	N+	30	NS	0.019
5. Duffy *et al.* (Dublin) *Clin Chem* 1991, **37**, 101.	48	331	Total	40	0.062	0.03†
6. Namer *et al.* (Nice) *Breast Cancer Res Treat* 1991, **19**, 85.	84	413	Total	35	–	0.02
			N+	35	0.03	0.008
7. Granata *et al.* (Milan) *Eur J Cancer* 1991, **27**, 970.	87	199	ER+ N-	40	0.02	0.01
8. Kute *et al.* (Winston Salem) *Cancer Res* 1991, **32**, 164.	29	165	N-	62	0.02	0.003
9. Pujol *et al.* (Montpellier) Submitted	60	123	Total	20	0.015	NS
			N+		0.015	0.009

*In arbitrary units; †univariate analysis.
Men., menopausal; NS, not significant; N, axillary lymph nodes; All studies (except nos 1, 3 and 9) have been performed with a similar double-determinant immunoassay using D7E3 and MIG8 monoclonal antibodies and ELISA (1 and 10) or IRMA (Elsa cath-D kit) quantification. Study 3 quantified only the mature form 34K by western blot using polyclonal antibodies. Study 1 and 9 used a less sensitive ELISA than the commercial kit, giving median value of 20.
Total number of patients with clinical follow up: 2020.

identified it as pro-cathepsin D [2]. Two monoclonal antibodies to this protein obtained from MCF7 cells (M1G8 and D7E3) have been used to develop a sandwich double-determinant immunoassay to measure the total concentration of the enzyme, including procathepsin-D (52 kD), the intermediate form (48 kD) and mature form (34 kD + 14 kD) [3].

Molecular and cell biology studies have shown that this enzyme is able to proteolyse several substrates including basement membrane and precursors of other proteinases if it is activated in an acidic micro-environment [4], and to stimulate breast cancer cell growth *in vitro* [5].

This enzyme is generally overexpressed in breast cancer cells under oestrogen stimulation in ER-positive cells and constitutively overexpressed in ER-negative cells [2]. Several centres well trained in steroid receptor assays, starting from tumour or cytosol banks, have carried out retrospective clinical studies which revealed that high cathepsin D concentration in breast cancer cytosol is associated with increased risk of developing metastasis [6–8].

A recent prospective study on 123 patients from the Cancer Center of Montpellier confirms these results, even though the prognostic value according to the Cox model was only significant in node-positive patients. Currently, at least 10 different clinical studies have shown that cathepsin-D status is a significant prognostic variable, even when associated with the most potent classical parameters such as node invasiveness (Table 1).

CATHEPSIN D ASSAY FOR CLINICAL ROUTINE OR CLINICAL RESEARCH USE?

A few major criteria should be considered in introducing a new prognostic marker for routine clinical use (Ingle and NIH Consensus Panel 1991). Cathepsin D fulfills most of these criteria.

First, cathepsin D is generally independent of other markers (steroid receptors, S-phase, node invasiveness, histological grade and tumour size) and, therefore, provides additional information.

Secondly, its predictive value has been validated in clinical studies performed independently in several different countries (Table 1). The fact that its prognostic value is, according to studies, more significant in node-negative or node-positive patients is not understood. It might be due to differences in the adjuvant therapy protocol or in the genetic or nutritional environment of the population.

Thirdly, it provides information on one biological aspect of tumours. Cathepsin D appears to be more correlated with metastasis than with local invasion or cell proliferation.

Fourthly, an immunoassay is commercially available, easy to perform and reproducible with satisfactory quality control. In this journal, T. J. Benraad *et al.* recently reported the first quality control of the cathepsin D assay by the EORTC study group [9]. They concluded that the commercially available cathepsin D kit (Elsa-cath-D, Cis Bio-International) is reliable with a low coefficient of variation. Further clinical evaluation of this new prognostic marker should thus be facilitated. One major advantage is that cathepsin D can be assayed in cytosol prepared for steroid receptor assays. It can, therefore, be of practical use in all laboratories set up to perform receptor assays. Interestingly, the pS2 protein, also induced by oestrogen in ER-positive breast cancer but not in ER-negative breast cancer, has an opposite prognostic significance since it is correlated with well differentiated tumours of better prognosis.

OPEN QUESTIONS ON CLINICAL AND BIOLOGICAL SIGNIFICANCE

We believe that there is now sufficient clinical and biological evidence to begin using cathepsin D in the routine staging of breast cancer. This is mostly due to the high practicality and reliability of the assay, to the potency and nature of the information which complements that given by the oestrogen and progesterone receptors assayed in the same cytosol extract. The fact that this information will be definitively lost if not performed on the primary tumour also argues in favour of a routine staging. How the different prognostic factors are interpreted in treatment decision has been previously discussed [1]. However, as for many other prognostic factors routinely used including the oestrogen receptor and histological grade, there are still several points which require clarification and further clinical and biological studies.

Clinical use from a practical viewpoint

Firstly, the best cut-off level varied according to the studies and it was not clear whether to consider the median value as around 40–50 pmoles/mg protein or a higher value (70 pmoles/mg protein) which corresponds to about 25% of patients with very high cathepsin-D levels.

Secondly, while all studies indicated a worse prognosis in high cathepsin-D tumours, it is not yet clear whether adjuvant therapy will be efficient in these high cathepsin-D tumours. Indeed, cathepsin-D might be associated with chemoresistance [10]. This might explain the discrepancies in its significance which were mostly seen in node-negative patients or only in node-positive patients according to studies (Table 1). There is, therefore, a need for well-controlled randomised clinical studies to test the actual benefit of this predictive marker for patients.

Thirdly, with the increasing use of mammography screening for breast cancer, smaller tumours should also be staged. Reliable and quantitative immunohistochemical and cytochemical assays will be required to replace cytosolic assays, and they will have to be validated as is currently being done for receptor assays [11].

Fourthly, since cathepsin D is subject to very complex regulation involving not only oestrogen, but also growth factors and oncogenes [12], its value should be tested in other solid tumours able to metastasize.

Cathepsin D expression and metastatic ability

The biological significance of the correlation between high cathepsin D expression and increased metastatic ability is not yet clear. Several questions are currently being addressed.

Firstly, since the total concentration of the enzyme is assayed, is the mature enzyme or its precursor the most potent marker? A specific assay of pro-cathepsin D using an antibody specific to the pro-enzyme [13] might provide an answer to this question.

Secondly, since the cytosol contains proteins from several types of cells, which type of cells are actually overexpressing cathepsin D in patients? *In situ* localisation of the protein by immunohistochemistry and of the mRNA by *in situ* hybridisation suggests that tumoral cells are mostly responsible for high cytosolic concentrations (our unpublished studies). Participation of macrophages, which also produce cathepsin-D, is generally low and depends on their proportion in the tumour.

Thirdly, is cathepsin D a consequence or a cause of metastasis? There is evidence in favour of both explanations. Since the cathepsin D gene is also induced by growth factors, it might be the consequence of a defect in the complex regulation of its gene expression. Alternatively, cathepsin D is a protease whose routing and processing is altered in cancer cells and, thus, it might be actively engaged in the metastatic process, as suggested by the increased metastatic ability of tumour cells which overexpress cathepsin D following transfection of its cDNA [14].

CONCLUSIONS

Clinical use of new prognostic markers which can be assayed on all tumours (including smaller ones) appears to be essential for treatment decisions. Since the aggressiveness of solid tumours depends on the concentration and the nature of molecular markers located in the primary tumours, such tumours should be extensively staged at the molecular level in order to obtain information on proliferation, differentiation, hormone responsiveness and invasiveness of this tumour. This staging can only occur once at surgery and would not be generally feasible thereafter to guide treatment decisions.

The cost of this initial staging is relatively low compared to the wide-spread and repeated assay of circulating markers which is generally performed too late when curative therapy is no longer possible. However, for economic and practical reasons, it is necessary to choose from an increasing number of these new markers.

The relative potency of new biochemical markers should, therefore, be evaluated in multiparametric studies that include several of these markers. Moreover, since most published studies are retrospective, there is a need for randomised prospective studies in which the efficiency of adjuvant therapy is investigated relative to cathepsin D status. Priority should be given to markers which can be assayed in the same samples, for instance, in cytosol which gives information on the hormone responsiveness (ER and PR, or pS2) and aggressiveness (cathepsin D) of tumour.

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Papers

Evidence for Individual Differences in the Radiosensitivity of Human Skin

Susan L. Tucker, Ingela Turesson and Howard D. Thames

Previously published clinical data have been re-analysed to investigate individual differences in the radiosensitivity of human skin. In the clinical studies, acute and late skin reactions were recorded for 254 breast cancer patients receiving radiotherapy to the internal mammary nodes following simple or modified radical mastectomy. Each patient was treated bilaterally with different fractionation schedules to the right and left fields. Patients were assigned prospectively to 10 different treatment groups of 11–35 patients each, with all patients in a group receiving the same pair of fractionation schedules to the right and left fields. In the present study, correlations between the skin reactions in the two treatment fields per patient were investigated. For each of three different endpoints—peak reflectance measure of erythema, peak acute skin reaction score, and a ranking measure of the progression rate of telangiectasia—significant correlations were found between the levels of skin injury to the right and left treatment fields of the patients in most treatment groups. Although there were correlations between the absorbed doses in the right and left fields, statistical analyses indicated that dose effects were not sufficient to explain fully the patient-to-patient differences in skin response. Thus, these data provide evidence for the existence of individual differences in the radiation response of human skin, both for early and late effects. Whether these differences are dominated by heterogeneity in intrinsic cell radiosensitivity or by other factors has yet to be determined. However, there was no clear evidence of a correlation between the acute and late endpoints, suggesting that the individual differences in radiosensitivity are not dominated by a common genetic component expressed equally in all cells.
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INTRODUCTION

DURING THE past decade it has been recognised that human tumours differ in their intrinsic cell radiosensitivity and that *in vitro* radiosensitivity is correlated with clinical radioresponsiveness [1–3]. Heterogeneity in intrinsic tumour-cell sensitivity exists even among tumours of the same histologic type [4–7]. This recognition has led to the development of radiosensitivity assays that, it is hoped, will help to predict the response to radiotherapy of the individual tumour [4, 7–9].

More recently, attention has turned to the possibility that differences in normal tissue response among radiotherapy patients may also be due, at least in part, to differences in intrinsic cell sensitivity. It has been known for some time that individuals with ataxia telangiectasia, for example, are hypersensitive to radiation [10], but there may also be differences in inherent radiation sensitivity among apparently normal individuals.

The purpose of this paper is to present evidence for individual differences in the radiosensitivity of human skin, based on a reanalysis of previously published data. Since 1972, prospective studies of acute and late skin reactions have been carried out in

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