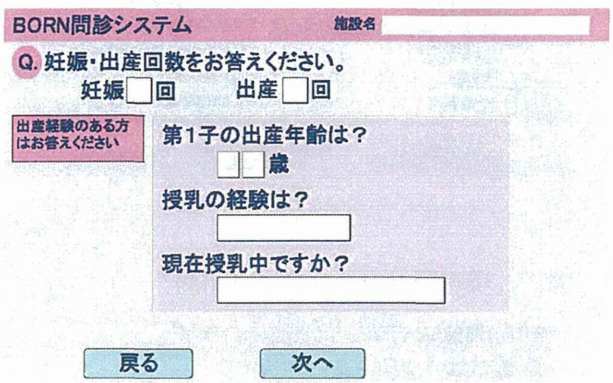
妊娠・出産に関する情報も、化学療法剤を使用する場合には欠かせない情報である（図９）。

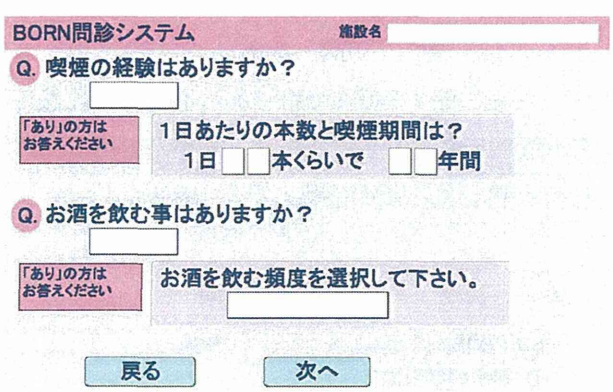
図９　妊娠・出産に関する質問



１０）　喫煙・飲酒に関する質問

喫煙は、抗がん剤の肺毒性発現のリスク因子であり、重要な情報となる。また、飲酒は肝疾患を伴うことが多いので必要な情報の一つとなる（図１０）。

図１０　喫煙・飲酒に関する質問



１１）　乳がんの家族歴

乳がんの家族歴は、患者の乳がんという疾患に対する受け止め方に大きく影響を及ぼすので，必要な情報となる。特に、抗がん剤の治療を受けた家族の介護を経験したかどうかは、抗がん剤の効果や副作用軽減に関わる因子となる可能性がある。卵巣癌やその他の癌に対しても同様の抗がん剤が使用されるので、その経験は副作用軽減に関わる可能性がある。

図１１　乳がんの家族歴

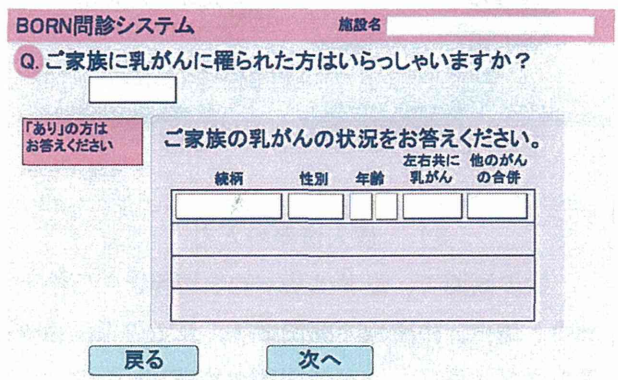
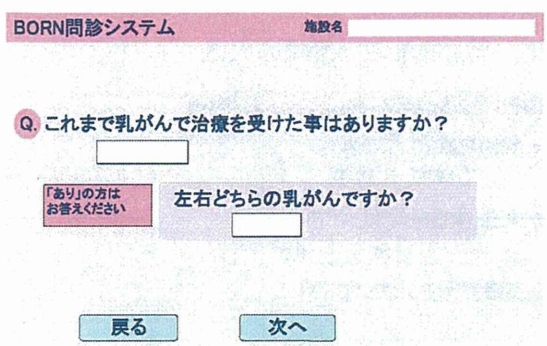


図１２　乳がんの治療歴



１２）　乳がんの治療歴

当然のことながら、乳がんの治療歴は、抗がん剤効果予測と毒性軽減には必須の情報である（図１２）。この問診システムは初診時を想定しているので、乳癌に対する手術の既往歴を含めて詳しい情報はこのシステムから外れて個別に取ることになる。

１３）　卵巣がん・子宮がん，その他の既往歴

卵巣がん、子宮がんやその他の既往がある場合、抗がん剤治療を受けている可能性があり、抗がん剤の毒性軽減の関わる必要な情報となる（図１３）。その他の癌は、食道がん、胃がん、大腸がん、肺がん、肝臓がん、胆嚢がん、胆管がん，膵がん、腎がん、前立腺がん、白血病、悪性リンパ腫，その他とし、その発症年齢とともに聴取する（図１４）。

図１３　卵巣がん・子宮がんの既往

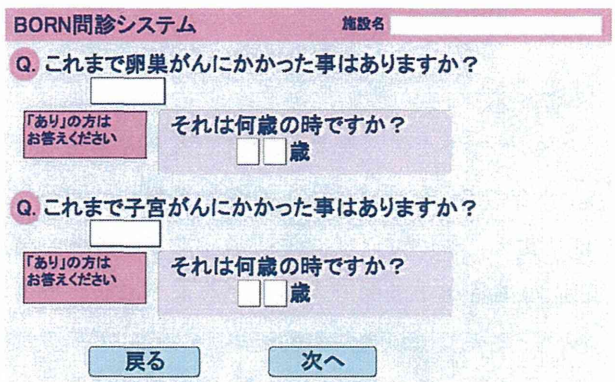
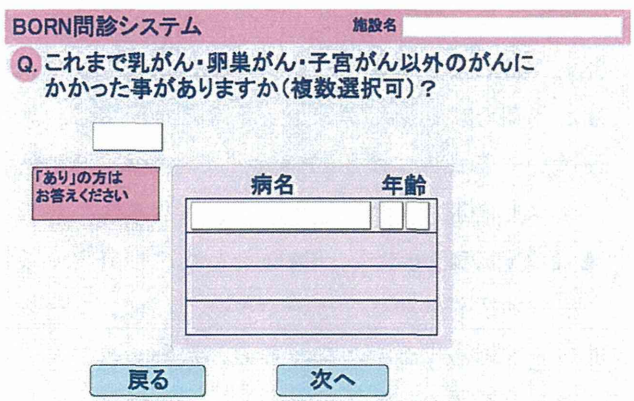


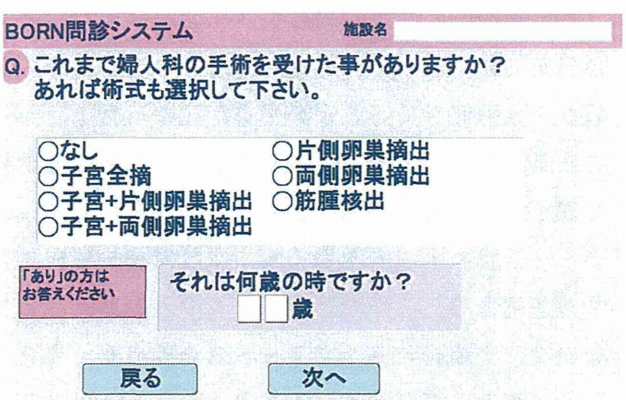
図１４　その他のがんの既往



１４）　婦人科手術の既往

婦人科手術の既往は内分泌療法を行う時に患者の女性ホルモン状態に関わる情報となる（図１５）。

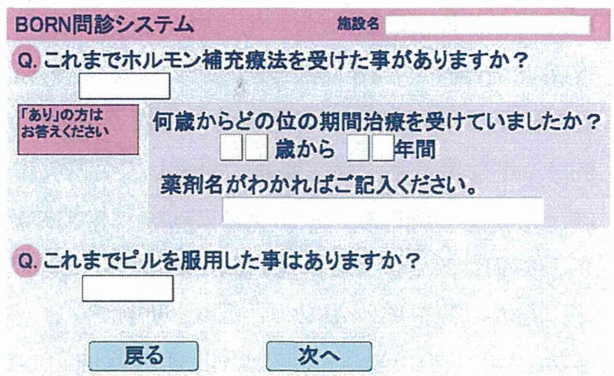
図１５　婦人科手術の既往



１５）　ホルモン補充療法とピル

内分泌療法を行う時の患者の女性ホルモン状態に影響を与える閉経後のホルモン補充療法の生むと閉経前の避妊用ピルの使用歴について聴取する（図１６）。

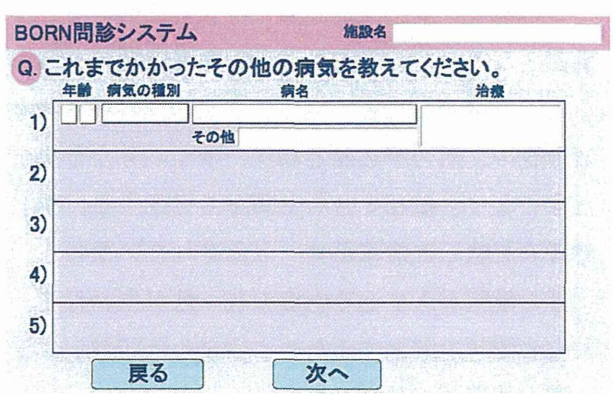
図１６　ホルモン補充療法とピルの使用歴



１６）　その他の疾患の既往歴

がん以外の疾患の既往歴については、患者の併存疾患として現在の病態と治療状況についての情報と捉えると、抗がん剤効果予測や毒性軽減に影響を与える因子となる。既往歴をアレルギー、肝、眼科、血液、縦隔・胸膜、循環器、消化器、腎、精神、代謝異常、胆・膵、脳・神経、肺、婦人科の種別にわけ、それぞれの種別の主要な疾患を網羅して主要な疾患は選択できるようにした（図１７）。選択の対象に選んだ疾患以外の疾患についてはその他の部分にキーボードを用いて直接入力することにした。

図１７　その他の疾患の既往歴



D　考察

患者の基本的な医療情報である身長と体重は、初診時には聴取されることの少ないと思われるが、患者の身体状態を知るための重要な情報であり、BMI を計算するために必要である。抗がん剤効果予測や毒性軽減に大きく関わる因子が患者の女性ホルモン状態であるので、初潮、月経状態、閉経状態、妊娠・出産、さらには婦人科手術歴、ホルモン補充療法の治療歴、避妊用ピルの使用歴を加えた。また、嗜好品では抗がん剤の肺毒性のリスク因子と考えられる喫煙、肝障害を伴う可能性のある飲酒を加えた。抗がん剤効果予測や毒性軽減に関連してものの中では、乳がんの既往歴が最も重要であり、まず確認をし、詳細はこのシステムを離れて聴取することになる。乳がん以外のがんについては、卵巣がん、子宮がん、およびその他のがんを個別に聴取項目とした。この場合も既往がある場合には、個別に抗がん剤の使用状況を聴取する必要がある。がん以外の疾患の既往歴については、併存疾患として現在の病態と治療状況を把握するという観点から重要であるので、疾患を種別にわけ、その中の主要な疾患については網羅して、的確に情報が得られるようにした。がんの家族歴では、家族の癌の治療過程から副作用発現の遺伝的な素因の可能を検討するため必要な情報となる。

通常の診療においては予診用紙などを用いて患者自ら記載する形式をとることが多いので、量的に聴取できる項目が限られ、必要な項目に記入漏れが多くみられる。欠損値が多いと集学的治療アルゴリズムの構築と意思決定過程の定式化に用いることが困難となり、抗がん剤効果予測や毒性軽減を解析することも困難となる。さらに、データベース化するには改めて入力が必要となり，多くの労力を要するだけでなく、転記ミスなど問題も発生する。特に、患者の記載した情報がコード化されていないと、入力者の解釈が入り込み内容の統一性が失われる。これらの問題を解決するために開発した問診システムの基本的なコンセプトは、

① 患者が容易に入力できるもの

② 入力はすべて数字またはカテゴリー化された選択肢で行えるもの。

③ 入力され、電磁的になった医療情報のセキュリティーが守られるもの

④ 修正や確認が容易にできるもの

である。

コンセプト１の「患者が容易に入力できるもの」については、タッチパネルの端末を使い、すべて画面に触れるだけで入力できるようにし、かつ、基本的に１項目を１画面で入力できるようにし、内容の多いところでは複数の画面を用い、分けて入力できるようにし、ちょうどページをめくるように入力をしていけるようにした。コンセプト２については入力する医療情報はほとんど全てカテゴリー化された内容をとし、数値もドロップダウンなどの機能を使用して選択できるようにし、キーボードなしで入力できるようにした。コンセプト３については、患者が入力する端末である iPAD からホストの PC の共有ファイルを開いて入力するので、入力された医療情報は iPAD 内にはなく、iPAD とともにデータが遺漏するリスクをさけることができる。さらに、Ｉ患者、Ｉファイルとすることで、患者が入力するファイルにほかの患者のデータが入っていない環境とする。コンセプト４については、各画面で修正、前画面に戻ることができるようにし、また、入力終了後は、入力内容をリストにしてプリントアウトし、患者自らが入力内容を確認ができるようにした。

E　結論

Retrospective にこの問診システムに今まで初診の問診票で得られたデータを入力してみると、多くの項目で欠損値がでることが明らかになったことから、この問診ステムを用いることで患者の医療情報の量や質が大幅に改善できると期待され、そこで得られるデータは抗がん剤効果予測や毒性減弱の解析に資すると考える。この問診システムの入力画面は２２なので、Ｉ画面の入力に１５～３０秒必要とすると入力にかかる時間は６～１１分ということになり、外来の待ち時間で入力が可能と思われる。この問診システムの有用性と患者の負担の程度を検証するた

めに臨床研究を計画している。

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書

がん薬物療法の副作用軽減と医療経済学的効果

研究分担者
石黒　洋　京都大学医学部附属病院・京大病院がんセンター　特定講師

A. 研究の要旨

抗精神病薬の制吐剤としての使用

悪心・嘔吐に対しプロクロルペラジンやオランザピンなどの抗精神病薬がしばしば使用されている。高リスクレジメンにおいて、プロクロルペラジンと5HT3 拮抗薬併用を対照群の治療としたプラセボ対照ランダム化比較試験で、新規制吐剤であるアプレピタントやパロノセトロンはその有効性を示せていない（図１参照）。 アプレピタントとオランザピンを比較したランダム化比較試験でも、制吐効果は同等で悪心に関してはオランザピンが優れていた。しかし既に後発品が発売されている抗精神病薬の場合、悪心・嘔吐に対する有効性評価を目的にプラセボ対照ランダム化比較試験を行うことは困難であり、ガイドラインでの強い推奨には至っていない。

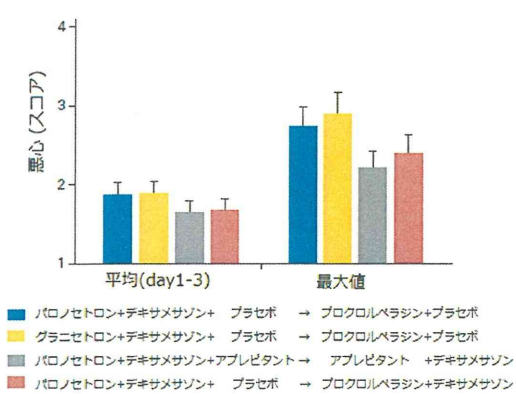


図１　高リスクレジメンで、プロクロルペラジン使用下ではパロノセトロン（青 vs. 黄）やアプレピタント（灰 vs. 茶）はその有効性を示せなかった。

１コース当りの薬剤費は抗がん治療のAC療法で約2万円、FEC療法は約4万円、制吐剤のアプレピタントは1万1200円そしてパロノセトロンは1万4500円、一方オランザピンは2000円(10mg/日, day1-4)、プロクロルペラジンは120円(30mg/日, day2-3)と非常に安価である 。悪心・嘔吐高リスクの患者で、前述の臨床試験に準じパロノセトロンとデキサメサゾンに併用する薬剤をアプレピタントからオランザピンに変更するだけで、1サイクル当り約1万円の薬剤費削減になる（図2参照）。日本全体で考えると、アントラサイクリン系薬剤の使用が想定される原発性乳癌再発高リスク患者での薬剤費が年間約4億円削減出来る計算である。

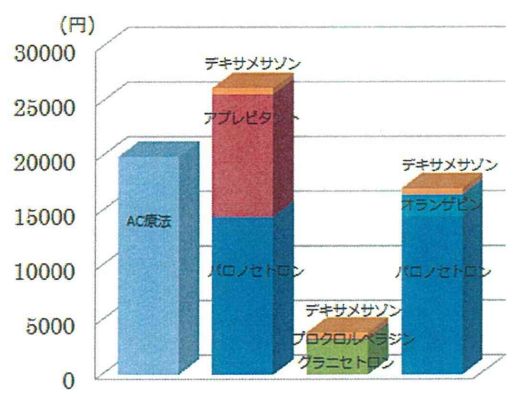


図２　１サイクルあたりの薬剤費

PEG G-CSF

長期作用型G-CSF(国内では承認申請中)の用量は、海外では体格に関わらず固定用量(6mg)で処方されている。一方、海外で長期作用型G-CSFの投与を受けた日本人女性における有害事象などの当院における詳細な検討から、小柄な日本人女性においては欧米人と同量の用量(6mg)では過量投与となり有害である可能性も示唆された。日本人において用量反応性を調べたランダム化第II相試験でも、一段階下の3.6mg が日本人における推奨用量であることが示さ

れた。長期作用型G-CSFの有効性を検証し承認申請を目的としたプラセボ対照ランダム化第III相比較試験では、日本人の推奨用量における高い有効性が検証された。

B: 結論

抗精神病薬の制吐剤としての使用

非常に薬価が低く後発品も発売されている抗精神病薬を使用した制吐療法は、その有効性において最新の制吐療法に勝るとも劣らず、かつ費用対効果が非常に優れていることを腫瘍学領域で最も影響の大きいジャーナルにて紙上発表した。今後、制吐療法のガイドラインなどに取り入れられれば、発展途上国など医療財政の乏しい地域における極めて有益な情報となる。

PEG G-CSF

体格に関わらない固定用量で処方される長期作用型G-CSF製剤は、体格の小さい女性では過量投与であることが示唆された。より多くの臨床腫瘍医に認知してもらうことが必要と考え、臨床医学領域で最も影響の大きいジャーナルにて紙上発表した。体格に応じた用量を選択できれば有害事象の軽減のみならず医療費の軽減が期待できる。

C. 研究発表

1. 論文発表

Ishiguro H, Kawaguchi K, Nishimura T, Toi M.
Antipsychotics-containing regimen as an alternative to standard anti-emetics for delayed nausea induced by highly emetogenic chemotherapy.
J Clin Oncol: Vol.31, No.10, p1377-8, 2013.4.1, Epub 2013 Feb 19

Ishiguro H, Toi M.
Body size may matter when determining pegfilgrastim dosage. *N Engl J Med:* Accepted for publication

2. 学会発表

なし。

D. 知的財産権の出願・登録状況

1. 特許取得

なし。

2. 実用新案登録

なし

3. その他

なし

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書

抗がん剤効果予測による乳がん患者の再発リスク抑制と毒性軽減および医療経済負担軽減に関する検証的研究

分担研究者　近藤　正英　筑波大学准教授

研究要旨

原発性乳癌術後化学療法の適応に関して、費用対効果も含めたガイドライン作りで主導的な立場にある英国の National Institute for Health and Clinical Excellence（NICE）で、既存のコンセンサスガイドラインなどで再発リスクが中等度の症例に限って、21 遺伝子シグネチャ（Onco*type*DX®）の検査の使用を薦める勧告案が検討されている。本研究では、わが国の保険診療への NICE 勧告に準じた形での導入に関して費用対効果を検討した。結果は、LN−症例を対象とした場合と、LN±症例を対象とした場合の増分費用効果比はそれぞれ、¥305,414/QALY と¥600,863/QALY となった。これらは医療の技術革新に対する社会的支払い意思額の閾値¥5,000,000/QALY を下回り、21 遺伝子シグネチャの使用は費用対効果に優れることが明らかになった。経済性の観点からは、わが国でも NICE に準じた形での導入を支持することができる。

A．研究目的

ホルモンレセプター陽性早期乳がん患者において、再発リスクの評価と化学療法の効果予測に基づいて、術後化学療法の適応を示し、効果の見込めない化学療法を回避することによって、毒性軽減につながる多遺伝子検査（multigene assay）のなかで、国際的に実用化されている 21 遺伝子シグネチャ（Onco*type*DX®）の経済評価を国際的な診療ガイドライン開発の動向に対応して行った。

21 遺伝子シグネチャは、米臨床腫瘍学会（ASCO）や全米総合癌情報ネットワーク（NCCN）の臨床ガイドラインに組み込まれている治療方針決定のための遺伝子診断であるが、費用が高い（日本では 45 万円）ことが特徴のひとつである。ただし、たとえば、多遺伝子検査によらない従来の診断情報に基づいて、術後化学療法の適応とされた患者について、多遺伝子検査によって費用が高い（最大 100 万円程度）化学療法が回避される場合もあり、医療財源の効率的使用という観点からは、適切な経済評価が求められる。我々は、本研究班の平成 22 年度の分担研究としてこれを行い、公的医療制度でこの検査を提供することが社会的に受け入れられるものであることを示してきた（Kondo M et al (2011) Breast Cancer Res Treat. 127(3):739-49.）。

一方、国際的な診療ガイドライン開発のなかで、公的医療制度の中で医療技術の効果に加えて費用対効果も含めたガイドライン作りで主導的な立場にある英国の National Institute for Health and Clinical Excellence（NICE）での原発性乳癌術後化学療法の適応に関して、多遺伝子検査（multigene assay）を含む医療技術の総合的な検討が進み、既存のコンセンサスガイドラインなどで再発リスクが中等度の症例に限って、21 遺伝子シグネチャ（Onco*type*DX®）の検査を使用する勧告案がパブリックコメントを受け付ける段階まで進んでいる。

また、わが国でも平成 26 年度の診療報酬改定に向けて、中央社会保険医療協議会において医療技術

の保険診療への導入に際して費用対効果を考慮に入れる方法を検討する費用対効果評価専門部会が立ち上がり、NICE の勧告に準拠するような費用対効果も考慮に入れた保険適応の検討が求められるようになってきている。

そこで本研究では、わが国の現在の実地臨床で再発リスクが中等度と判断される症例に限って、21 遺伝子シグネチャ（Onco*type*DX®）の検査を使用する場合の費用対効果を明らかにすることを目的とした。

B．研究方法

日本での 21 遺伝子シグネチャの経済評価として研究分担者ら行った先行研究（Kondo M et al (2011) Breast Cancer Res Treat. 127(3):739-49.）の経済モデルを使用して、現在の実地臨床での再発リスク評価としてモデル化した St Gallen コンセンサスガイドラインで中等度と判断された症例のみに 21 遺伝子シグネチャを使用した場合の費用対効果を検討した。NICE の勧告案では、ER+、LN−、HER2−症例が対象であるが、LN±症例を対象にする場合も検討した。

（倫理面への配慮）

本研究は、文献による経済モデリングであり疫学研究の倫理指針や臨床研究の倫理指針にかかるものではない。ただし、経済評価の対象である医療技術は商業的に提供されているものである。そこでその商業的提供者に関して利益相反がないことを確認した。

C．研究結果

表 1 が費用対効果の検討結果である。LN−症例を対象とした場合と、LN±症例を対象とした場合のいずれでも、増分費用正、増分効果正となった。増分費用効果比はそれぞれ、¥305,414/QALY と ¥600,863/QALY となった。これらは医療の技術革新に対する社会的支払い意思額の閾値 ¥5,000,000/QALY を大きく下回った。つまり、21 遺伝子シグネチャの使用は極めて費用対効果に優れる。

表 1　再発リスク中等度症例への 21 遺伝子シグネチャ使用の費用対効果

適応		費用 (¥)	効果 (QALY)
LN−	検査無	3,627,193	19.48
	検査有	3,685,809	19.67
	増分	59,616	0.19
	増分費用効果比	*¥305,414/QALY*	
LN±	検査無	3,818,952	18.82
	検査有	3,911,744	18.97
	増分	92,791	0.15
	増分費用効果比	*¥600,863/QALY*	

D．考察

わが国で再発リスクが中等度と判断される症例に限って、21 遺伝子シグネチャ（Onco*type*DX®）の検査を保険診療に導入することは、経済性の観点から社会的に受け入れられると考えられる。

再発リスクに関わらず導入する場合の費用対効果が、増分費用効果比が、N－，Her2＋で ¥384,828/QALY、N±，Her2－で¥434,096/QALY、N±，Her2＋で¥568,533/QALY、N±，Her2－で ¥614,765/QALY であることと比較すると、再発リスクが中等度と判断される症例に限って導入することの効率性はほぼ同様である。

E．結論

乳癌術後患者での 21 遺伝子シグネチャ（Onco*type*DX®）の検査について、再発リスクが中等度の症例への使用が NICE によって勧告されると、わが国の保険診療への同様の形での導入の議論が始まると考えられる。本研究結果からは、そのような形での導入を支持することができる。

G．研究発表

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H．知的財産権の出願・登録状況（予定を含む）

1. 特許所得

なし。

2. 実用新案登録

なし。

3. その他

なし。

研究協力者

星淑玲　筑波大学研究員

Ⅲ. 研究成果の刊行に関する一覧表

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Endocrine-Related Cancer (2012) **19** 485–496

Oestrogen-induced genes in ductal carcinoma *in situ*: their comparison with invasive ductal carcinoma

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Abstract

It is well known that oestrogens play important roles in both the pathogenesis and development of invasive ductal carcinoma (IDC) of human breast. However, molecular features of oestrogen actions have remained largely unclear in pure ductal carcinoma *in situ* (pDCIS), regarded as a precursor lesion of many IDCs. This is partly due to the fact that gene expression profiles of oestrogen-responsive genes have not been examined in pDCIS. Therefore, we first examined the profiles of oestrogen-induced genes in oestrogen receptor (ER)-positive pDCIS and DCIS (DCIS component (DCIS-c)) and IDC (IDC component (IDC-c)) components of IDC cases ($n=4$ respectively) by microarray analysis. Oestrogen-induced genes identified in this study were tentatively classified into three different groups in the hierarchical clustering analysis, and 33% of the genes were predominantly expressed in pDCIS rather than DCIS-c or IDC-c cases. Among these genes, the status of *MYB* (C-MYB), *RBBP7* (RBAP46) and *BIRC5* (survivin) expressions in carcinoma cells was significantly higher in ER-positive pDCIS ($n=53$) than that in ER-positive DCIS-c ($n=27$) or IDC-c ($n=27$) by subsequent immunohistochemical analysis of the corresponding genes ($P<0.0001$, $P=0.03$ and $P=0.0003$ respectively). In particular, the status of C-MYB immunoreactivity was inversely ($P=0.006$) correlated with Ki67 in the pDCIS cases. These results suggest that expression profiles of oestrogen-induced genes in pDCIS may be different from those in IDC; and C-MYB, RBAP46 and survivin may play important roles particularly among oestrogen-induced genes in ER-positive pDCIS.

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Introduction

Breast cancer is the most common malignant neoplasm in women worldwide. In particular, the incidence of ductal carcinoma *in situ* (DCIS) has been markedly increasing possibly due to advancements in population-based mammographic screening for detection (Li *et al.* 2005), and ~20% of breast carcinoma cases actually present as pure DCIS (pDCIS) without invasive components at the time of diagnosis in many countries (Kepple *et al.* 2006, Tsikitis & Chung 2006). This pDCIS is in general considered as a precursor lesion of invasive ductal carcinoma (IDC). It has been demonstrated that approximately half of untreated pDCIS progresses to IDC with marked variability in the latency of the progression (Cuzick 2003) and up to 80% of IDC were also reported to contain at least small foci of DCIS component (DCIS-c) distinct from the IDC component (IDC-c) if carefully evaluated (Ellis *et al.* 2003). Therefore, it has become very important to examine the biological features of pDCIS to identify the possible molecular mechanisms related to the acquisition of invasive

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properties and subsequently to improve clinical outcome of early breast cancer patients.

It is well known that oestrogens play important roles in the progression of breast carcinoma through an interaction with oestrogen receptor (ER). ER is expressed in approximately two-thirds of IDC, and endocrine therapy has been administered in these patients in order to suppress the intratumoural oestrogen actions. A great majority of pDCIS was also reported to express ER in their parenchymal cells (Wiechmann & Kuerer 2008), and the results of National Surgical Adjuvant Breast Project (NSABP) B-24 trial did demonstrate that adjuvant tamoxifen therapy was clinically effective in ER-positive pDCIS and reduced the recurrence of noninvasive breast carcinomas by 27% (Cuzick 2003). Pathological and biological responses to preoperative tamoxifen therapy in ER-positive pDCIS patients has been also reported (Chen *et al.* 2009).

ER is well known to activate the transcription of various target genes in a ligand-dependent manner, and various oestrogenic functions are also characterised by expression profiles of these genes in oestrogen target cells. Various oestrogen-responsive genes have been also identified in IDC (Frasor *et al.* 2003), and an analysis of these genes can greatly contribute to the understanding of molecular functions of oestrogen actions, such as cell proliferation, anti-apoptosis, invasion, metastasis, recurrence and resistance to endocrine therapy, in IDC (Suzuki *et al.* 2012). However, expression profiles of oestrogen-responsive genes have not necessarily been examined in pDCIS to the best of our knowledge. Therefore, it has still remained unclear whether oestrogen actions and/or effectiveness of endocrine therapy in pDCIS could be the same as that in IDC.

Therefore, in this study, we first examined expression profiles of oestrogen-induced genes in carcinoma tissues of breast cancer patients and demonstrated different expression profiles of oestrogen-induced genes in ER-positive pDCIS from ER-positive DCIS-c or IDC-c following an isolation of the corresponding cells under light microscopy using laser-capture dissection. Subsequent microarray analysis indicated that *MYB* (C-MYB), *RBBP7* (retinoblastoma suppressor (Rb)-associated protein 46 (RBAP46)) and *BIRC5* (survivin) were predominantly expressed in pDCIS compared with DCIS-c and IDC-c among these oestrogen-induced genes. Therefore, we subsequently immunolocalised these gene products in ER-positive pDCIS tissues in order to further characterise their oestrogenic actions.

Materials and methods

Patients and tissues

Two sets of tissue specimens were used in this study. The first set is composed of eight specimens of ER-positive breast carcinoma (four pDCIS and four IDC cases) obtained from Japanese women (age: 51–77 years in pDCIS, and 49–75 years in IDC) who underwent surgical treatment from 2003 to 2008 in the Department of Surgery, Tohoku University Hospital, Sendai, Japan. One IDC patient was premenopausal, and the others were postmenopausal. All the IDC specimens used in this study contained both DCIS-c and IDC-c, and the patients did not receive chemotherapy, irradiation or hormonal therapy before the surgery. All the cases examined in this study were associated with nuclear grade 1 or 2, and their ER labelling index (LI) was ranged from 40 to 96% in pDCIS, 35 to 100% in DCIS-c and 42 to 100% in IDC-c respectively. These specimens were stored at −80 °C for subsequent microarray analysis. The second set is composed of 80 specimens of ER-positive ductal carcinoma of human breast (53 pDCIS and 27 IDC cases) obtained from Japanese female patients who underwent surgical treatment from 1995 to 2008 in the Department of Surgery, Tohoku University Hospital, Sendai, Japan. These patients also did not receive chemotherapy, irradiation or hormonal therapy before the surgery. The median age of these patients was 61 years (range 39–80 years) for pDCIS and 55 (range 32–84 years) for IDC, and all the cases of IDC contained both DCIS-c and IDC-c in this study. All the specimens were fixed in 10% formalin and embedded in paraffin wax.

The entire resected surgical specimen was sectioned into slices with 3–5 mm thickness, and all the slices were histologically evaluated by surgical pathologists. In this study, pDCIS was defined when DCIS-c was detected but no foci of stromal invasion in carcinoma were detected in all the slides of the cases evaluated. In the first set, thinner section stained with haematoxylin and eosin was prepared from the frozen specimen, and histological features of these lesions were confirmed.

Research protocols for this study were approved by the Ethics Committee at Tohoku University Graduate School of Medicine (accession no. 2009-107).

Laser-capture microdissection/microarray analysis

Gene expression profiles of breast carcinoma cells in the first set of the specimens (four pDCIS, four DCIS-c and four IDC-c samples) were examined using microarray analysis. Laser-capture microdissection

(LCM) was conducted using the MMI Cellcut (Molecular Machines and Industries, Flughofstrase, Glattbrugg, Switzerland). Briefly, breast carcinomas were embedded in Tissue-Tek optimal cutting temperature compound (Sakura Finetechnical Co., Tokyo, Japan) and sectioned at a thickness of 10 μm. Breast carcinoma cells were dissected under the light microscopy and laser transferred from these frozen sections. The total RNA (~200 ng) was subsequently extracted from these cell fractions isolated by LCM using the RNeasy Micro Kit (Qiagen). In IDC cases, carcinoma cells were separately collected in DCIS-c and IDC-c. Whole Human Genome Oligo Microarray (G4112F (ID: 012391)), Agilent Technologies (Waldbronn, Germany), containing 41 000 unique probes, was used in this study, and sample preparation and processing were performed according to the manufacturer's protocol. In this study, we focused on the expression of 51 genes identified to be oestrogen-induced ones in MCF7 breast carcinoma cells by Frasor *et al.* (2003) (two genes corresponding *PPP2R1B* were included in this analysis). Hierarchical clustering analysis was performed using the Cluster and TreeView programs (the software copyright Stanford University 1998–1999, http://rana.stanford.edu) to generate tree structures based on the degree of similarity, as well as matrices comparing the levels of expression of individual genes in each specimens.

Immunohistochemistry

Immunohistochemical analysis was performed in the second set (53 pDCIS and 27 IDC cases) described above. Monoclonal antibodies for ER (6F11), progesterone receptor (PR; 1A6) and Ki67 (MIB1) were purchased from NovoCastra (Newcastle upon Tyne, UK), Chemicon (Temecula, CA, USA) and DAKO (Carpinteria, CA, USA) respectively. Rabbit polyclonal antibodies for human epidermal growth factor receptor-2 (HER2; A0485) were obtained from DAKO. In addition, rabbit polyclonal antibodies for C-MYB (EPR718(2)), RBAP46 (EPR5082) and survivin (NB500-201) were purchased from Epitomics (Burlingame, CA, USA) and Novus Biologicals (Littleton, CO, USA) respectively.

A Histofine Kit (Nichirei Biosciences, Tokyo, Japan) that employs the streptavidin–biotin amplification method was used in this study. Antigen retrieval was performed by heating the slides in an autoclave at 120 °C for 5 min in antigen retrieval solution (pH 9.0; Nichirei Biosciences) for C-MYB immunostaining or citric acid buffer (2 mM citric acid and 9 mM trisodium citrate dehydrate (pH 6.0)) for immunostaining of other antibodies. Dilutions of primary antibodies used in this study were as follows: ER, 1/50; PR, 1/50; HER2, 1/100; Ki67, 1/100; C-MYB, 1/50; RBAP46, 1/1000 and survivin, 1/1000. The antigen–antibody complex was subsequently visualised with 3,3′-diaminobenzidine (DAB) solution (1 mM DAB, 50 mM Tris–HCl buffer (pH 7.6) and 0.006% H_2O_2) and counterstained with haematoxylin. As a positive control, human IDC tissue was used for C-MYB (McHale *et al.* 2008) and survivin (Barnes *et al.* 2006) immunostaining, and a cellblock of MCF7 breast carcinoma cells was used for RBAP46 (Creekmore *et al.* 2008). Normal rabbit IgG was used instead of the primary antibody, as a negative control in this study.

Immunohistochemical evaluation

Immunoreactivity of ER, PR and Ki67 was detected in the nucleus, and their immunoreactivity was evaluated in counting more than 1000 carcinoma cells for each case. The percentage of immunoreactivity, i.e. LI, was subsequently determined. Cases with ER LI of more than 1% were considered ER-positive breast carcinoma in this study (Hammond *et al.* 2010). HER2 immunoreactivity was evaluated according to the grading system proposed in HercepTest (DAKO), and strongly circumscribed membrane-immunoreactivity of HER2 present in more than 30% carcinoma cells were considered positive (Wolff *et al.* 2007). Both C-MYB and RBAP46 immunoreactivities were detected in the nuclei of carcinoma cells and were evaluated by employing the H-scoring system (McCarty *et al.* 1985). Briefly, C-MYB- and RBAP46-positive carcinoma cells were classified into three groups according to immunointensity (i.e. strongly, moderately or weakly positive cells), and H scores were subsequently generated by adding together 3×% of strongly positive cells, 2×% of moderately positive cells, 1×% weakly positive cells, and 0×% of negative cells (range 0–300). Survivin immunoreactivity was detected in the cytoplasm of carcinoma cells and was semi-quantitatively evaluated by modified H-scoring system (Mehta *et al.* 2012), in which the percentage of cytoplasmic immunoreactivity was categorised as 0 (no expression), 10 (up to 10%), 20 (10–20%) until 100 (90–100%), and giving a possible range of 0–300.

Statistical analysis

An association of various clinicopathological factors among three carcinoma components (pDCIS, DCIS-c and IDC-c) was evaluated using a Kruskal–Wallis test or a cross-table with the χ^2 test. An association between C-MYB, RBAP46 and survivin immunoreactivity and

clinicopathological factors was evaluated by a cross-table using the χ^2 test. An association of clinicopathological factors between two components of IDC cases was evaluated using a Wilcoxon signed-ranks test. The statistical analyses were performed using the JMP Pro version 9.02 (SAS Institute, Inc., Cary, NC, USA), and P values of <0.05 were considered significant in this study.

Results

Expression profiles of oestrogen-induced genes in pDCIS compared with those of DCIS-c and IDC-c

We first surveyed expression profiles of oestrogen-induced genes in isolated carcinoma cells of pDCIS using microarray analysis which was focused on oestrogen-induced genes reported by Frasor *et al.* (2003), in order to examine the characteristics of oestrogenic actions in pDCIS. Fifty-one oestrogen-induced genes examined were tentatively classified into three groups (i.e. Groups A, B and C) depending on the hierarchical clustering analysis (Fig. 1). In addition, isolated and examined pDCIS carcinoma cells were clustered among the cases examined. Results demonstrated that the genes in Group C were predominantly expressed in pDCIS rather than in DCIS-c or IDC-c, and the genes in Group A were predominantly expressed in DCIS-c and/or IDC-c. Genes classified into Group B were expressed regardless of the carcinoma types. No significant clustering of samples was detected in association with nuclear grade, menopausal status and ER LI of the cases examined in this study.

As shown in Table 1, no significant differences of characteristics were detected between Groups A and C in this study.

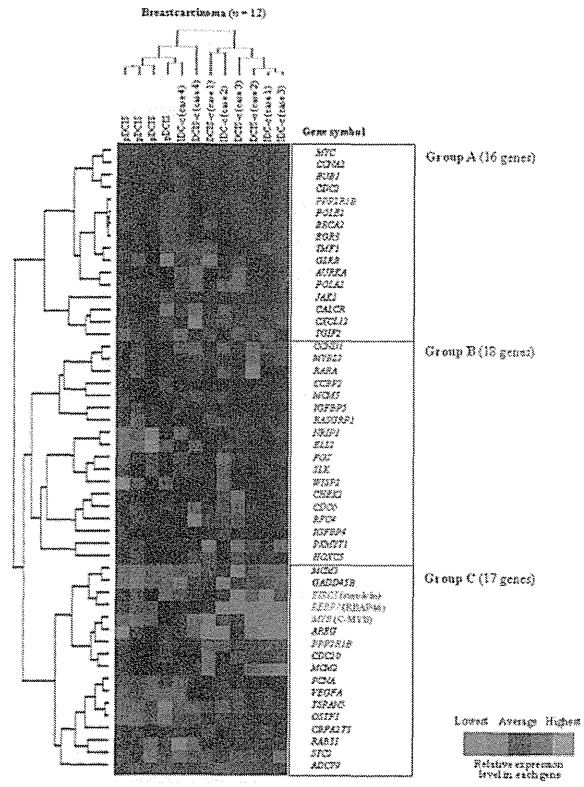

Figure 1 Hierarchical clustering analysis of mRNA expression levels focused on oestrogen-induced genes identified by Frasor *et al.* (2003). Colour of blocks represents relative mRNA expression level of each gene compared with the average in 12 breast carcinoma samples (four pDCIS, four DCIS-c and four IDC-c). Gene symbols in each gene were listed. Gene- performed immunohistochemistry was noted in red. Two genes corresponding *PPP2R1B* were coloured green.

Clinicopathological features of pDCIS, DCIS-c and IDC-c

We then evaluated an association of various clinicopathological parameters among pDCIS ($n=53$), DCIS-c ($n=27$) and IDC-c ($n=27$), which were examined in this study. Nuclear grade ($P=0.68$), ER LI ($P=0.94$), PR LI ($P=0.87$) and HER2 status ($P=0.33$) were not significantly different among these three groups, but Ki67 LI was significantly ($P<0.0001$) lower in pDCIS than that in DCIS-c and IDC-c (Table 2). No significant differences of patients' age ($P=0.43$) and menopausal status ($P=0.34$) were detected between pDCIS and IDC patients in this study. HER2 positive status in our study (45% in pDCIS, 33% in DCIS-c and 30% in IDC-c) was consistent with that of a previous report (Park *et al.* 2006).

Immunolocalisation of C-MYB, RBAP46 and survivin in pDCIS

Results of the microarray analysis demonstrate different expression profiles of oestrogen-induced genes in pDCIS compared with those in DCIS-c and IDC-c. We then performed immunohistochemistry for three representative oestrogen-induced genes (C-MYB (*MYB*), RBAP46 (*RBBP7*) and survivin (*BIRC5*)) in the breast carcinoma tissues in Group C towards further confirmation of the findings.

As demonstrated in Fig. 2A, C-MYB was immunolocalised in the nuclei of carcinoma cells, and its H-score was significantly ($P<0.0001$) higher in pDCIS than that in DCIS-c or IDC-c (Fig. 2B). RBAP46 immunoreactivity was also detected in the nuclei of carcinoma cells (Fig. 2C), and its immunoreactivity was significantly ($P=0.03$) higher in pDCIS (Fig. 2D).

Table 1 Comparison of characteristics of genes between Groups A and C

Characteristic of genes	Number of genes: Group A ($n=15$)	Number of genes: Group C ($n=16$)	*P* value
First time of significant upregulation by oestrogen			
4 h	7 (47%)	11 (69%)	0.51
8 h	1 (7%)	0 (0%)	
24 h	5 (33%)	4 (25%)	
48 h	2 (13%)	1 (6%)	
Major biological function			
Cell cycle and apoptosis	6 (40%)	5 (31%)	
Growth factors, cytokines and hormones	1 (7%)	3 (19%)	
Receptors and signal transduction proteins	2 (13%)	5 (31%)	0.34
Transcription factors and transcriptional coregulators	6 (40%)	3 (19%)	

Data of characteristics of genes were taken from a report by Frasor *et al.* (2003). Data are presented as the number of cases and percentage. Two genes corresponding *PPP2R1B* were excluded in this table, because these were classified into both Groups A and C.

Survivin was immunolocalised in the cytoplasm of carcinoma cells, and some nuclei of the carcinoma cells were also immunohistochemically positive for survivin (Fig. 2E). Relative survivin immunoreactivity was significantly ($P=0.0003$) higher in pDCIS than that in DCIS-c or IDC-c (Fig. 2F).

As shown in Table 3, when we divided the cases into two groups according to several important pathological factors, such as nuclear grade, HER2 status and ER LI, C-MYB immunoreactivity was significantly higher in pDCIS than that in DCIS-c or IDC-c regardless of the status. Similar tendency was also detected in RBAP46 and survivin immunoreactivities; but *P* values did not reach significant levels in some groups.

As two genes corresponding *PPP2R1B* were classified into different groups (i.e. Groups A and C) in the microarray analysis (Fig. 1), we performed immunohistochemistry of PPP2R1B (also known as a protein phosphatase 2, regulatory subunit A, β (PP2A-Aβ)) in these cases. PPP2R1B immunoreactivity was detected in the breast carcinoma cells (Supplementary Figure S1A, see section on supplementary data given at the end of this article), but its immunointensity was generally weak and was not significantly different among the pDCIS, DCIS-c and IDC-c groups examined in this study (Supplementary Figure S1B, see section on supplementary data given at the end of this article).

Association between C-MYB, RBAP46 and survivin immunoreactivity and various clinicopathological parameters in pDCIS

Results of both microarray and immunohistochemical analyses described earlier indicated that C-MYB, RBAP46 and survivin were abundantly expressed in pDCIS. As demonstrated in Table 4, when 53 pDCIS cases examined were tentatively classified into two different groups according to the median value of C-MYB H-score, the status of C-MYB immunoreactivity was inversely ($P=0.006$) associated with Ki67 LI in pDCIS cases. No other significant association was detected between C-MYB immunoreactivity and other clinicopathological parameters of the patients examined, such as patients' age, menopausal status, nuclear grade, comedo necrosis, ER LI, PR LI and HER2 status. The status of RBAP46 immunoreactivity was not significantly associated with any clinicopathological parameters examined (Table 5), while the status of survivin immunoreactivity was positively associated with patients' age ($P=0.002$; Table 6). Association between PPP2R1B immunoreactivity and clinicopathological parameters in pDCIS cases is summarised

Table 2 Association of various clinicopathological parameters among pDCIS, DCIS-c and IDC-c

Parameter	pDCIS ($n=53$)	DCIS-c ($n=27$)	IDC-c ($n=27$)	*P* value
Nuclear grade[a]				
Grades 1+2	44 (83%)	24 (89%)	24 (89%)	0.68
Grade 3	9 (17%)	3 (11%)	3 (11%)	
ER LI (%)	81 (12–100)	80 (15–100)	80 (8–100)	0.94
PR LI (%)	40 (0–100)	40 (0–100)	40 (0–100)	0.87
HER2 status[a]				
Negative	29 (55%)	18 (67%)	19 (70%)	0.33
Positive	24 (45%)	9 (33%)	8 (30%)	**<0.0001**
Ki67 LI (%)	4 (1–12)	8 (1–23)	12 (1–32)	

P value <0.05 was considered significant and is in boldface.
[a]Data are presented as the number of cases and percentage. All other values represent the median (min–max).

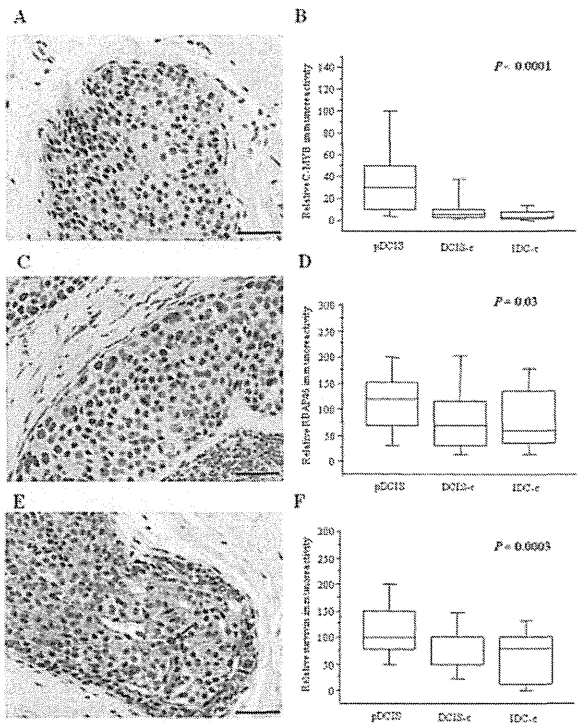

Figure 2 Immunohistochemistry for C-MYB (A and B), RBAP46 (C and D) and survivin (E and F) in the breast cancer cases. Immunoreactivity of C-MYB (A) and RBAP46 (C) was detected in nuclei of carcinoma cells in pDCIS. Survivin was immunolocalised in the cytoplasm of carcinoma cells in pDCIS and was also positive in some nuclei of the carcinoma cells (an arrow; E). Bar=50 μm respectively. Relative immunoreactivity of C-MYB, RBAP46 and survivin in pDCIS, DCIS-c and IDC-c was summarised in B, D and F respectively. Data are represented as box and whisker plots. Briefly, the median value is represented by a horizontal line in each box, and the 75th (upper margin) and 25th (lower margin) percentiles of the values are demonstrated. The upper and lower bars indicate the maximum and minimum values respectively. In F, the median value of relative survivin immunoreactivity in DCIS-c was 100. Statistical analysis was carried out using the Kruskal–Wallis test. *P* values <0.05 were considered significant and were indicated in bold letter.

in Supplementary Table S1, see section on supplementary data given at the end of this article.

Association between clinicopathological parameters and three oestrogen-induced proteins in DCIS-c and IDC-c

As summarised in Table 7, Ki67 LI was significantly lower ($P=0.04$) in DCIS-c than that in IDC-c, but no significant differences between clinicopathological parameters of the patients and the status of immunoreactivity of C-MYB, RBAP46 and survivin were detected between DCIS-c and IDC-c of 27 IDC patients in this study.

Discussion

pDCIS is generally considered as a precursor lesion of IDC. Two different models have been proposed to explain the possible mechanisms of transition from pDCIS to IDC, i.e. theories of linear progression or parallel disease (Wiechmann & Kuerer 2008). In the former model, low-grade pDCIS lesions are considered to progress to high-grade pDCIS lesions and then to become IDC (Carter *et al.* 1988, Bodian *et al.* 1993, Lakhani *et al.* 1999). In the latter model of hypothesis, low-grade pDCIS lesions progress to low-grade IDC and high-grade pDCIS lesions to high-grade IDC (Sontag & Axelrod 2005, Wiechmann & Kuerer 2008). Accumulating data including chromosomal-alteration studies support the parallel disease theory (Hwang *et al.* 2004, Irvine & Fentiman 2007), and the great majority of molecular alterations detected in breast carcinoma, including *ESR1* which codes for ER, can be clearly detected already in pDCIS, whether high or low grades (Nofech-Mozes *et al.* 2005, Burkhardt *et al.* 2010). In this study of ER-positive breast carcinoma, both ER and PR LIs in pDCIS were similar to those in IDC-c or DCIS-c, which is considered to be compatible with parallel disease theory of development. Shibuya *et al.* (2008) also previously demonstrated that various oestrogen-producing enzymes were abundantly expressed in pDCIS, and intratumoural oestrogen concentration was similar between pDCIS and IDC (Shibuya *et al.* 2008). Therefore, oestrogens are considered to play pivotal roles in pDCIS as well as in IDC.

Results of our present study also demonstrated that Ki67 LI was significantly lower in ER-positive pDCIS than that in ER-positive IDC. Antibody Ki67 recognises cells located in all the phases of cell cycle except for G_0 (resting) phase (Gerdes *et al.* 1983), and Ki67 LI is closely correlated with the cell proliferation activity of the tissues (van Diest *et al.* 2004). Ki67 was also reported as a prognostic factor in pDCIS (van Diest *et al.* 2004) as well as in IDC (de Azambuja *et al.* 2007), and increased Ki67 was associated with negative ER status of breast carcinoma (Burkhardt *et al.* 2010). All these findings suggest that oestrogen actions are more associated with cell proliferation of breast carcinoma in IDC than in pDCIS.

This is the first study to demonstrate expression profiles of oestrogen-induced genes in pDCIS compared with IDC. Results of our present microarray analysis did reveal that one-third of oestrogen-induced genes were predominantly expressed in pDCIS, while the other one-third of the genes mainly in IDC and the rest in both categories with equivalent frequency.

Table 3 Statistical associations of C-MYB, RBAP46 and survivin immunoreactivity among pDCIS, DCIS-c and IDC-c cases according to several pathological parameters

Parameter	C-MYB immunoreactivity	RBAP46 immunoreactivity	Survivin immunoreactivity
Nuclear grade			
Grades 1+2	**<0.0001**	**0.04**	**0.001**
Grade 3	**0.008**	0.5	0.3
HER2 status			
Negative	**<0.0001**	**0.02**	**0.01**
Positive	**0.01**	0.73	**0.02**
ER LI (%)			
8–79	**0.0003**	0.06	**0.01**
80–100	**0.0002**	0.20	**0.008**

Data are presented as *P* values. *P* values <0.05 were considered significant and are in boldface.

These findings suggest that oestrogenic actions in pDCIS were different from those in IDC, even if the carcinoma cells expressed ER and intratumoural oestrogen was present at a significant level in both of these lesions. Among the genes predominantly expressed in IDC (Group A in Fig. 1), *EGR3* (early growth-responsive gene 3) was reported to play a pivotal role in the process of oestrogen-mediated invasion in breast cancer, and its expression was associated with adverse clinical outcome of the patients with ER-positive IDC (Suzuki *et al.* 2007). In addition, the kinetochore-bound protein kinase *BUB1* (budding uninhibited by benzimidazoles 1) is also considered to play possible role in the process of breast tumourigenesis (Klebig *et al.* 2009), and its mRNA expression was also reported to be positively associated with clinical recurrence in ER-positive IDC patients (Suzuki *et al.* 2012). *MYC* (C-MYC) was also reported to be associated with poor prognosis or adverse clinical outcome of ER-positive breast cancer patients (Chen & Olopade 2008). Robanus-Maandag *et al.* (2003) reported that *MYC* amplification may drive transition from pDCIS to IDC in human breast (Robanus-Maandag *et al.* 2003), although some conflicting data were reported in the literature (Burkhardt *et al.* 2010). These findings suggest that oestrogen-mediated transactivation is considered to vary among the target genes, and the genes promoting aggressive biological or clinical behaviour of breast carcinoma cells may be more efficiently induced by oestrogen in IDC. However, immunoreactivity of C-MYB, RBAP46 and survivin was not associated with ER LI in pDCIS cases in this study, and previous studies have demonstrated that the expression of these molecules was regulated by several factors (for instances, miroRNA-150 downregulated C-MYB in liver cancer stem cells (Zhang *et al.* 2012), RBAP46 functioned as a downstream target gene of WT1 (Guan *et al.* 1998), and genetic variants of the survivin promotor were associated with survivin expression (Xu *et al.* 2004)). Therefore, factors other than oestrogen may also be involved in the different expression profiles of oestrogen-induced genes in pDCIS from IDC. Our experiments serve as a starting point for clarifying the molecular features of oestrogen actions in pDCIS, and further examination is required.

We first identified C-MYB, RBAP46 and survivin as oestrogen-induced proteins predominantly expressed in pDCIS compared with IDC in this study. Among these three genes identified by gene profilings, a nuclear transcription factor C-MYB regulates differentiation and proliferation in various types of cells (Oh & Reddy 1999), and expression of *C-MYB* mRNA was

Table 4 Association between C-MYB immunoreactivity and clinicopathological parameters in pDCIS

	C-MYB immunoreactivity		
Parameter	High (*n*=26)	Low (*n*=27)	*P* value
Patients' age	61 (48–80)	61 (39–80)	0.91
Menopausal status[a]			
Premenopausal	7 (30%)	3 (56%)	0.14
Postmenopausal	19 (70%)	24 (44%)	
Nuclear grade[a]			
Grades 1+2	20 (77%)	24 (89%)	0.25
Grade 3	6 (23%)	3 (11%)	
Comedo necrosis[a]			
Absent	11 (42%)	7 (26%)	0.21
Present	15 (58%)	20 (74%)	
ER LI (%)	84 (13–100)	80 (12–100)	0.77
PR LI (%)	40 (6–93)	46 (0–100)	0.72
HER2 status[a]			
Negative	14 (54%)	15 (56%)	0.90
Positive	12 (46%)	12 (44%)	
Ki67 LI (%)	3 (1–10)	6 (2–12)	**0.006**

Fifty-three pDCIS cases were classified into two (i.e. high and low) groups according to the median value of C-MYB immunoreactivity. *P* value <0.05 was considered significant and is in boldface.
[a]Data are presented as the number of cases and percentage. All other values represent the median (min–max).

Table 5 Association between RBAP46 immunoreactivity and clinicopathological parameters in pDCIS

Parameter	RBAP46 immunoreactivity		*P* value
	High (n=28)	Low (n=25)	
Patients' age	65 (39–80)	54 (49–77)	0.06
Menopausal status[a]			
Premenopausal	4 (14%)	6 (24%)	0.81
Postmenopausal	24 (86%)	19 (76%)	
Nuclear grade[a]			
Grades 1+2	21 (75%)	23 (92%)	0.99
Grade 3	7 (25%)	2 (8%)	
Comedo necrosis[a]			
Absent	9 (32%)	9 (36%)	0.77
Present	19 (68%)	16 (64%)	
ER LI (%)	88 (12–100)	80 (13–100)	0.60
PR LI (%)	44 (6–100)	40 (0–100)	0.19
HER2 status[a]			
Negative	16 (57%)	13 (52%)	0.71
Positive	12 (43%)	12 (48%)	
Ki67 LI (%)	4 (1–12)	4 (2–10)	0.31

Fifty-three pDCIS cases were classified into two (i.e. high and low) groups according to the median value of RBAP46 immunoreactivity.
[a]Data are presented as the number of cases and percentage. All other values represent the median (min–max).

rapidly stimulated by oestrogen administration in the MCF7 breast carcinoma cells (Frasor *et al.* 2003). C-MYB protein was detected in ER-positive IDC and was associated with a good prognosis in the patients (Guerin *et al.* 1990, Drabsch *et al.* 2007, Deisenroth *et al.* 2010, Thorner *et al.* 2010). Immunohistochemistry for C-MYB in pDCIS has been reported only by McHale *et al.* (2008) to the best of our knowledge, in which C-MYB immunoreactivity in the breast carcinoma containing both pDCIS and IDC was significantly higher than that in normal/hyperplastic epithelium. Results of our present study first demonstrated that C-MYB immunoreactivity was significantly higher in pDCIS than in IDC and was inversely associated with Ki67 LI in pDCIS. Very recently, Thorner *et al.* (2010) reported that stable RNAi knock-down of endogenous *C-MYB* in the MCF7 cells increased tumourigenesis, both *in vitro* and *in vivo*, suggesting a tumour suppressor function in luminal breast cancer subtypes (Thorner *et al.* 2010). Results of our present study are consistent with these previously reported studies, and decreased induction of C-MYB expression by oestrogen may result in the possible acceleration of oestrogen-mediated cell proliferation of breast carcinoma in IDC.

RBAP46, a nuclear protein, was originally identified as histone-binding proteins and its components of protein complexes have been demonstrated to be involved in the process of histone deacetylation and chromatin remodelling (Zhang *et al.* 1997, Bowen *et al.* 2004). *RBAP46* mRNA expression was reported to be rapidly induced by oestrogens in MCF7 cells (Frasor *et al.* 2003). Results of previous *in vitro* studies demonstrated that RBAP46 modulated oestrogen responsiveness in MCF7 cells in a gene-specific manner through interaction with ERα (Creekmore *et al.* 2008), and RBAP46 was also reported to inhibit an oestrogen-stimulated progression of transformed breast epithelial cells (Zhang *et al.* 2007). However, immunohistochemical evaluation of RBAP46 has not been reported in breast carcinoma to the best of our knowledge. In this study, RBAP46 immunoreactivity was more frequently detected in ER-positive pDCIS than in IDC, which also indicated that RBAP46 may play an important role in the alteration of oestrogen actions in the process of transition from pDCIS to IDC.

Survivin is known as an inhibitor of apoptosis, which prevents cell death by mainly blocking activated caspases (Ryan *et al.* 2006). Survivin mRNA expression was reported to be slowly induced by oestrogen in MCF7 cells (Frasor *et al.* 2003). Immunolocalisation of cytoplasmic survivin has been reported in human breast carcinoma by several groups, with positivity ranging from 56 to 76% of pDCIS cases (Barnes *et al.* 2006, Okumura *et al.* 2008) and 17 to

Table 6 Association between survivin immunoreactivity and clinicopathological parameters in pDCIS

Parameter	Survivin immunoreactivity		*P* value
	High (n=25)	Low (n=28)	
Patients' age	66 (48–80)	54 (39–80)	**0.002**
Menopausal status[a]			
Premenopausal	4 (16%)	6 (21%)	0.61
Postmenopausal	21 (84%)	22 (79%)	
Nuclear grade[a]			
Grades 1+2	19 (76%)	25 (89%)	0.20
Grade 3	6 (24%)	3 (11%)	
Comedo necrosis[a]			
Absent	7 (28%)	11 (39%)	0.39
Present	18 (72%)	17 (61%)	
ER LI (%)	87 (27–100)	80 (12–100)	0.25
PR LI (%)	47 (0–100)	40 (7–100)	0.58
HER2 status[a]			
Negative	12 (48%)	17 (61%)	0.35
Positive	13 (52%)	11 (39%)	
Ki67 LI (%)	4 (1–12)	4 (1–12)	0.80

Fifty-three pDCIS cases were classified into two (i.e. high and low) groups according to the median value of survivin immunoreactivity. *P* value <0.05 was considered significant and is in boldface.
[a]Data are presented as the number of cases and percentage. All other values represent the median (min–max).