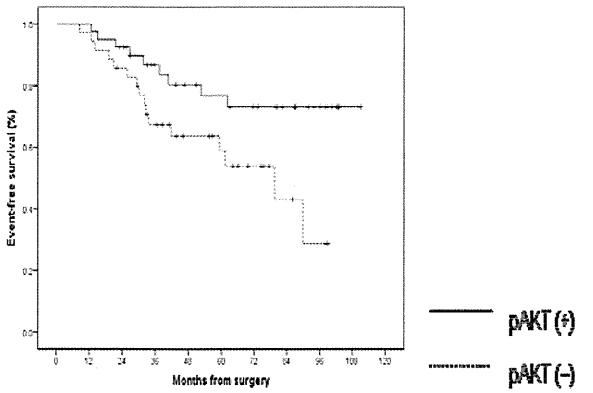
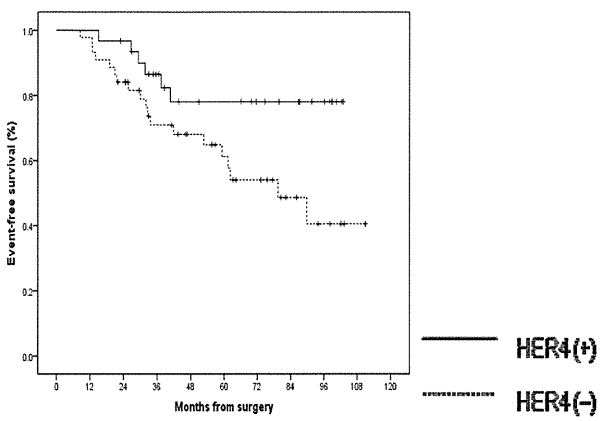
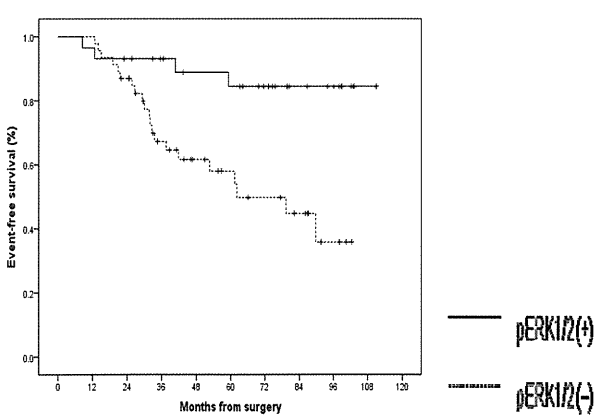




pERK1/2とTNBCの予後との関係

pERK1/2の発現量の有無別のEFSとOSを示す。



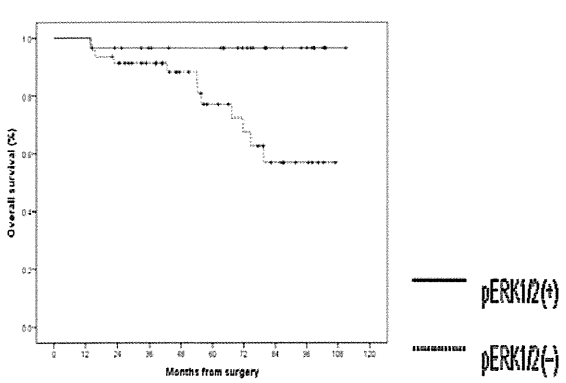
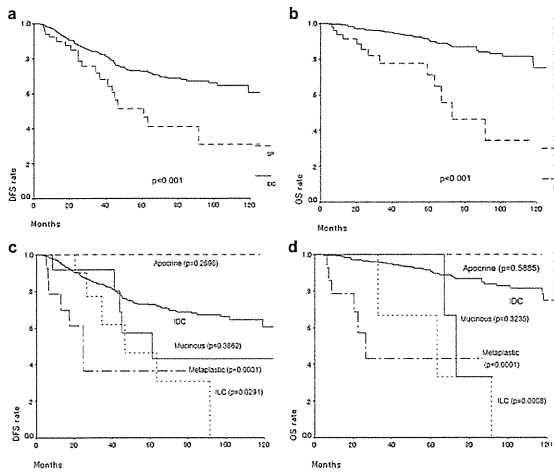


表４　解析対象（組織型）

解析対象（組織型）	症例数	%
B. Malignant (Carcinoma)		
a. Invasive carcinoma	500	89.0
a1. Papillotubular carcinoma	126	22.4
a2. Solid-tubular carcinoma	202	35.9
a3. Scirrhous carcinoma	172	30.6
b. Special types	62	11.0
b1. Mucinous carcinoma	12	2.1
b2. Medullary carcinoma	0	0
b3. Invasive lobular carcinoma	29	5.2
b4. Adenoid cystic carcinoma	0	0
b5. Squamous cell carcinoma	5	0.9
b6. Spindle cell carcinoma	4	0.7
b7. Apocrine carcinoma	5	0.9
b8. Carcinoma with cartilaginous and/or osseous metaplasia	1	0.2
b9. Tubular carcinoma	0	0
b10. Secretory carcinoma	1	0.2
b11. Invasive micropapillary carcinoma	1	0.2
b12. Matrix-producing carcinoma	4	0.7
b13. Others	0	0

図３ 浸潤性乳管がん（IDC）と他の亜型との生存期間の比較



トリプルネガティブ乳がんに特徴的なmetaplastic carcinomaや小葉がんの予後が有意に短かった。

表５　患者背景 (N=365)

	No. of patients (%) (N = 369)
Age	
≤50	146 (40)
>50	223 (60)
Menopause	
Premenopausal	152 (41)
Postmenopausal	217 (59)
Invasive tumor size (cm)	
≤2.0	276 (75)
>2.0 to ≤5.0	89 (24)
>5.0	4 (1)
Histology	
Invasive ductal carcinoma (IDC)	302 (82)
Mucinous carcinoma	26 (7)
Invasive lobular carcinoma	22 (6)
IDC with predominantly intraductal component	17 (4)
Others (medullary, tubular)	2 (1)
Ki-67 (≥10 %)	
Positive	221 (60)
Negative	148 (40)
Ki-67 (≥14 %)	
Positive	163 (44)
Negative	206 (56)
Ki-67 (≥20 %)	
Positive	87 (24)
Negative	282 (76)
Histological grade	
1	104 (28)
2	175 (47)
3	88 (24)
Unknown	2 (1)
Nuclear grade	
1	156 (42)
2	103 (28)
3	109 (30)
Unknown	1 (0)
Lymphovascular invasion	
Positive	138 (37)
Negative	230 (62)
Unknown	1 (0)
Adjuvant chemotherapy	
Yes	153 (41)
CMF	100 (65)[a]
UFT	52 (34)[a]
CEF	1 (1)
No	217 (59)
Hormone therapy (tamoxifen)	
Yes	187 (51)

	No. of patients (%) (N = 369)
No	182 (49)

表 6:Ki-67 の３つのカットオフと組織学的グレードの関係

Ki-67 labeling index	Number of tumors (%)								
	Total	Histological grade			p value	Nuclear grade			p-値
		1	2	3		1	2	3	
Low (<10 %)	148	61 (41)	73 (49)	14 (10)	<0.0001	98 (66)	33 (22)	17 (12)	<0.0001
High (≥10 %)	221[a]	43 (19)	102 (47)	74 (33)		58 (26)	70 (32)	92 (42)	
Low (<14 %)	206[b]	82 (40)	102 (50)	21 (10)	<0.0001	126 (61)	52 (25)	28 (14)	<0.0001
High (≥14 %)	163[c]	22 (13)	73 (45)	67 (41)		30 (18)	51 (31)	81 (50)	
Low (<20 %)	282[b]	97 (34)	144 (51)	40 (14)	<0.001	146 (52)	81 (29)	55 (19)	<0.0001
High (≥20 %)	87[c]	7 (8)	31 (36)	48 (55)		10 (11)	22 (25)	54 (62)	
Total	369[a]	104	175	88		156	103	109	

乳がん 369 症例を対象に、国内における Ki-67 の標準化を試みた。10, 14, 20%の３つのカットオフを設定し解析した結果、いずれのカットを用いても、組織学的悪性グレードと DFS に強い相関をみとめた。

D．考察

HER4の発現量、及び、AKTのリン酸化(pAKT) ERKのリン酸化(pERK)は、日本人のトリプルネガティブ乳がん (TNB) において、強い予後良好因子であることが明らかとなった。PI3CA変異は35%と高頻度であるのに比較して、AKT1変異を１例もみとめなかった。このことは、西洋人種とアジア人種とでは、主となる遺伝子異常が異なる可能性が示唆された。

トリプルネガティブ乳がんの亜型の中で、metaplastic carcinomaや小葉がんは予後不良で、従来の抗悪性腫瘍薬に対する感受性に乏しい。一方、アポクリン癌に関しては、従来の抗悪性腫瘍薬に対する感受性に乏しいが、予後は良好であった。国内乳がんにおいて、Ki-67の標準化が可能であったが、TNBCにおける使用は慎重に行うべきである。

E．結論

日本人のTNBで同一の治療法を受けた均質な集団における遺伝子解析の報告は少なく、今後のTNBの治療戦略の上で重要な基礎データとなる。PI3CA変異は、PI3K阻害剤、AKT阻害剤の薬剤感受性の予測マーカーとして既に報告されており、今後の創薬のキーとなる変異といえる。TNBの組織型の中でもアポクリン癌は緩やかな増殖を示し、他の亜型と区別する必要がある。Ki-67のバイオマーカー的価値は、Luminal A/Bに高く、TNBCにおける使用は慎重に行うべきであることが分かった。

F．健康危険情報

該当なし

G．研究発表

１．論文発表

1. Nagao T, Kinoshita T, Hojo T, Tsuda H, Tamura K, Fujiwara Y. The differences in the histological types of breast cancer and the response to neoadjuvant chemotherapy: the relationship between the outcome and the clinicopathological characteristics. Breast. 2012 21:289-295.

2. Ono M, Tsuda H, Yunokawa M, Yonemori K, Shimizu C, Tamura K, Kinoshita T, Fujiwara Y. Prognostic impact of Ki-67 labeling indices with 3 different cutoff values, histological grade, and nuclear grade in hormone-receptor-positive, HER2-negative, node-negative invasive breast cancers. Breast Cancer. 2013 Apr 13. [Epub ahead of print]

２．学会発表

なし

H．知的財産権の出願・登録状況（予定を含む。）

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（第３次対がん総合戦略事業）

平成 24 年度　分担研究報告書

分担研究課題名：TNB を対象とした大規模プロスペクティブ臨床試験

分担研究者：藤原　康弘

国立がん研究センター中央病院・企画戦略局長

研究要旨

TNBに対する前向き臨床試験より得られた乳がん組織、及び、末梢、血液検体を用い、抗悪性腫瘍薬の効果を規定するバイオマーカーの同定を試みた。１）原発性乳がん180症例を対象とした前向きコホート研究を施行した。Affymetrix gene Chip U133 plus 2.0を用いた30000 プローブを用いたマイクロアレイ解析の結果、pCRを予測する遺伝子群として、STAT, PML, WARS, CHKA が同定された。２）術前化学療法の前向き試験で得られた乳がん組織（65症例）を対象に、新規がん幹細胞マーカーであるNSのRNAの発現をreal time RT-PCR法を用いて測定した。NSのmRNA発現量が低いこととpCRとには、相関があり、多変量解析の結果でも臨床因子と独立した予測マーカーであった。HER2過剰発現のない乳がんに対する術前化学療法におけるCarboplatin/Weekly Paclitaxel→CEFとWeekly Paclitaxel→CEFのランダム化第II相試験（医師主導治験）の登録を終了した。現在、登録190症例に対して、約半数の100例の組織検体の確保が可能となった。BRCA1, PARP1, ZEB1, TWISTなど、DNA修復遺伝子や、がん幹細胞のマーカーについて免疫組織染色を施行した。Preliminaryな結果であるが、BRCA1の発現量が低いほうが、CBDCAの上乗せ効果が高い傾向にあった。現在、症例数と追加して検証を試みている。

A．研究目的

本研究班の目的の一つに、TNBを対象として、抗悪性腫瘍薬の効果を規定するバイオマーカーの同定がある。目的を達成するために、TNBに対する前向き臨床試験（プロスペクティブ試験）より得られた腫瘍検体（乳がん組織検体）、末梢血液検体を用いた、解析を行う。

B．研究方法

1）TNBの術前化学療法のpCRを予測する遺伝子群の探索（RNAを用いた30000 probeマイクロアレイ解析）

原発性乳がんを対象とした、術前化学療法の前向き試験で得られた乳がん組織（180症例）よりmRNAを抽出し、Affymetrix gene Chip U133 plus 2.0を用いたマイクロアレイ解析を施行した。ホルモン感受性、HER2発現量の有無によるサブセットによるクラスター解析を行った。又、トレーニングデータ120例に対し、8700 probeを用い、Wilcoxonのp値で順位付けを行う。その後、上位のprobe を使いテストデータ60例で判別の精度を評価した。評価法はSVM（線形判別式）を用い、判別式に使う遺伝子の個数は5-fold CVで決定した。

2）乳がん組織検体でのNS発現量の測定（RT-PCR, 免疫組織染色）

原発性乳がんを対象とした、術前化学療法の前向き試験で得られた乳がん組織（65症例）を対象に、新規がん幹細胞マーカーであるNSのRNAの発現をreal time RT-PCR法を用いて測定し、pCRとの相関を検討した。

3）HER2過剰発現のない乳がんに対する術前化学療法におけるCarboplatin/Weekly Paclitaxel→CEF Weekly Paclitaxel→CEFのランダム化第II相試験

（医師主導治験）

HER2過剰発現のない乳がんを対象に、標準的治療レジメンにCarboplatinの上乗せ効果を検証する比較試験（200例）の登録を終了した。付随研究の中で、乳がん検体の、BRCA1, PARP1, CHECK1, GNL3L, STAT, ZEB1, TWISTなどの発現量とPI3K変異を測定する。登録施設のほとんどにおいて付随研究のIRBが通過した。約半数の施設より未染の切片を回収した。登録症例数190例に対して100例の未染の切片が回収できた。

（倫理面への配慮）

本研究は、国立がん研究センター中央病院、若しくは、多施設共同研究に関しては、四国がんセンター、大阪医療センター、東京都立駒込病院、愛知がんセンター、神奈川県立がんセンターの各々の倫理委員会の承認を得て実施する。また測定施設・解析施設にあっても、施設内の倫理委員会の承認を得た後、測定を実施する。研究代表者らの研究グループは、既に乳がん患者を対象としたファルマコゲノミクス研究を経験しており、検体の匿名化や管理法などの具体的方法は熟知している。臨床検体の処理・管理は国立がん研究センター中央病院支援施設内で行う。

臨床材料に関しては、疫学研究に関する倫理指針に従い、患者から文書による同意を得た臨床材料を使用し、手術材料等の残余材料の研究利用については、診断等に一切の不都合を来さないこと、連結可能匿名化を行った後に解析を行うこと等を実施した。

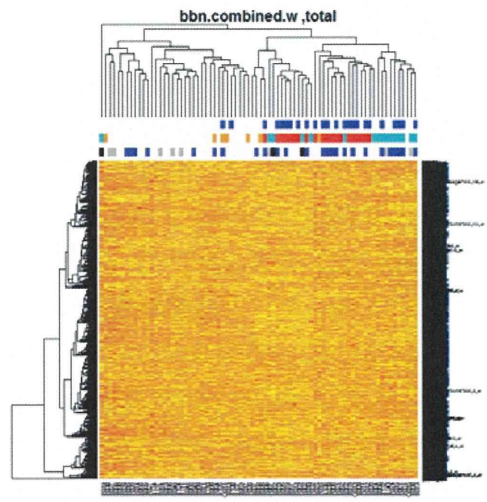
C．研究結果

1） TNBの術前化学療法のpCRを予測する遺伝子群の探索（マイクロアレイ解析）

トレーニングデータ120例、テストデータ60例の計180例の術前化学療法の症例を用いた。腫瘍検体にクラスタリングでは、比較的きれいにsubtype (enrich, HER2/Luminal B, Luminal A, TNB)に分かれることが確認できた（図1）。

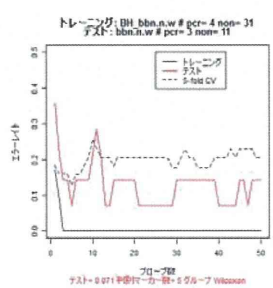
HER2陰性乳がんを対象とした判別解析では、pCRを予測する遺伝子群として、subtype（Luminal A に対しTNBであること）に加え、STAT, PML, WARS, CHKAなどが同定された（テストエラー7%:図2）。Gene Ontology解析、KEGG(Kyoto Encyclopedia of Genes and Genomes)のデータベース解析を行った。このことにより、HER2陰性乳がんに関しては、関連する遺伝子のパスウエイマップを作成することが可能であった（図3）。クラスタリング解析の精度を検証するため、トレーニングデータ（N=120）とテストデータ（N=60）を比較した。HER2陰性乳がんに関しては、トレーニングデータの発現パターンは、テストデータにて極めて忠実に再現された（図4）。

図１　腫瘍検体を用いたクラスタリング



pcr: 青, non-pcr: 白 enrich: 赤, her2LB: 橙, LA: 白, TN: 水色 cr: 青, pr: 白, sd: 灰, pd: 黒

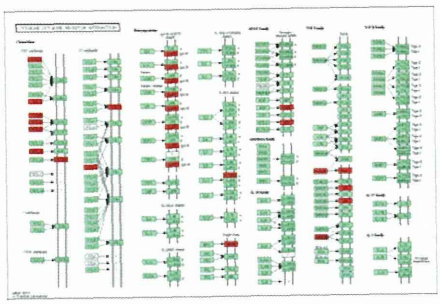
図２HER2陰性乳がんを対象とした判別解析



判別に用いるマーカー

1．Subtype（TN=1, LA=0） 2．STAT　3．PML 4．WARS 5．CHKA

図３パスウエイマップ（KEGGを用いた解析）



2) 乳がん組織検体でのNS発現量の測定

術前化学療法の前向き臨床試験より得られた乳がん組織45例を用いた。9例のpCRを得た。臨床背景との解析では、ホルモン陰性乳がんにおいて、陽性乳がんと比較してpCRが得られやすい結果となった。NSのmRNA発現量が低いこととpCRとには相関があった（図4）。多変量解析の結果、NSのmRNA発現量が低いことは、ホルモン受容体の有無、HER2受容体の有無、組織学的グレード、病期、腫瘍径から独立するpCRを予測する分子マーカーであった（表1）。NS対する特異抗体を用いてNSの乳がんでの発現量を免疫組織染色法により測定した。NSの免疫組織染色法によるタンパク発現量はHER2陰性乳がんで多く見られ、特に、TNBCにおいてはそれが顕著であった。又、同一サンプルにおける、NSのmRNA発現量とタンパク発現量はよく相関した。

図4 NSのmRNA発現量とpCRの相関

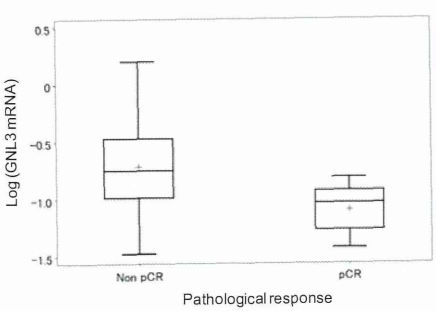


表1 pCRと相関する因子（多変量解析）

Variables		OR	95%CI		P
Log(GNL3)	1 unit gain	0.04	0.00	0.98	0.049
HR	negative	1			
	positive	0.12	0.01	2.48	0.171
HER2	negative	1			
	positive	0.38	0.05	3.10	0.366
Histological grade	1 or 2	1			
	3	1.73	0.07	44.18	0.742
Stage	I/IIA/IIB/IIIA	1			
	IIIB/IIIC/IV	2.17	0.14	32.84	0.576
Tumor size	<5	1			
	>5	0.19	0.01	2.74	0.220

3) TNBにおける白金製剤の有用性を検証と感受性を規定する分子マーカーの同定

HER2過剰発現のない乳がんに対する術前化学療法におけるCarboplatin/Weekly Paclitaxel→CEFとWeekly Paclitaxel→CEFのランダム化第II相試験（医師主導治験）の登録を終了した。登録190例の内、バイオマーカー研究解析可能症例は約100例である。Preliminaryな結果であるが、BRCA1の発現量が低いほうが、CBDCAの上乗せ効果が高い傾向にあった。現在、症例数と追加して検証を試みている。

D．考察

プロスペクティブ研究により、NS の mRNA・タンパク発現量が、TNB の pCR と強い相関があることがわかった。又、マイクロアレイ解析においても、HER2 陰性乳がんの pCR を予測する遺伝子として、STAT, PML, WARS, CHKA などが同定された。又、HER2過剰発現のない乳がんに対する術前化学療法における Carboplatin/Weekly Paclitaxel→CEF と Weekly Paclitaxel→CEF のランダム化第 II 相試験（医師主導治験）の登録が計画通り終了した。

E．結論

TNBの中で、pCRを予測する遺伝子群としてSTATなどのシグナル伝達系タンパク質が同定されたことは重要である。一方、NSを代表するがん幹細胞、EMTの特徴をもつTNBには、従来型抗がん剤（アンスラサイクリン系、タキサン系など）の感受性が少ない。さらに、BRCA1異常TNBCは、白金製剤に対する感受性が高い。又、Olaparibに代表されるPARP阻害剤の効果が期待できる。これらの前向き研究によるバイオマーカーの同定は今後のTNBの個別化医療に役立つであろう。

TNBを対象としたPARP阻害剤を用いた前向き研究（未承認薬PARP阻害剤：Olaparib（アストラゼネカ社）とエリブリン（エーザイ：2011年上旬、乳がん領域にて承認予定）の併用試験：医師主導治験）は、平成２５年１月に開始し順調に登録を進めている。

Ｆ．健康危険情報

該当なし

Ｇ．研究発表

１．論文発表

1. Tanabe Y, Hashimoto K, Shimizu C, Hirakawa A, Harano K, Yunokawa M, Yonemori K, Katsumata N, Tamura K, Ando M, Kinoshita T, Fujiwara Y. Paclitaxel-induced peripheral neuropathy in patients receiving adjuvant chemotherapy for breast cancer. Int J Clin Oncol. 2013 18:132-138.

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２．学会発表

Ｈ．知的財産権の出願・登録状況（予定を含む。）

１．特許取得

なし

２．実用新案登録

なし

３．その他

なし

III.　研究成果の刊行に関する一覧

別紙4

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
小泉史明.	第2章　がん臨床バイオマーカー	公益社団法人　日本薬理学会	実験薬理学実践治療薬	金芳堂	京都市	2012	45-51

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nagatsuma AK, Shimizu C, Takahashi F, Tsuda H, Saji S, Hojo T, Sugano K, Fujii H, Fujiwara Y.	Impact of recent parity on histopathological tumor features and breast cancer outcome in premenopausal Japanese women	Breast Cancer Res. Treat.	138(3)	941-950	2013
Ono M, Tsuda H, Yunokawa M, Yonemori K, Shimizu C, Ando M, Tamura K, Kinoshita T, Fujiwara Y.	Prognostic impact of Ki-67 labeling indices with three different cut-off values and histological and nuclear grades in hormone-receptor-positive, HER2-negative, node-negative invasive breast cancers	Breast Cancer	In press		
Kurebayashi J, Kanomata N, Shimo T, Yamashita T, Aogi K, Nishimura R, Shimizu C, Tsuda H, Moriya T, Sonoo H.	Marked lymphovascular invasion, progesterone receptor negativity and high Ki67 labeling index predict poor outcome in breast cancer patients treated with endocrine therapy alone.	Breast Cancer	In press		
Tanabe Y, Hashimoto K, Shimizu C, Hirakawa A, Harano K, Yunokawa M, Yonemori K, Katsumata N, Tamura K, Ando M, Kinoshita T, Fujiwara Y.	Paclitaxel-induced peripheral neuropathy in patients receiving adjuvant chemotherapy for breast cancer	Int J Clin Oncol	18	132-138	2013

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Nagao T, Kinoshita T, Hojo T, Tsuda H, Tamura K, Fujiwara Y.	The differences in the histological types of breast cancer and the response to neoadjuvant chemotherapy: the relationship between the outcome and the clinicopathological characteristics	Breast	21	289-295	2012
Yunokawa M, Koizumi F, Kitamura Y, Katanasaka Y, Okamoto N, Kodaira M, Yonemori K, Shimizu C, Ando M, Masutomi K, Yoshida T, Fujiwara Y, Tamura K	Efficacy of everolimus, a novel mTOR inhibitor, against basal-like triple-negative breast cancer cells	Cancer Sci	103	1665-1671	2012
Kondo S, Ueno H, Hashimoto J, Morizane C, Koizumi F, Okusaka T, Tamura K.	Circulating endothelial cells and other angiogenesis factors in pancreatic carcinoma patients receiving gemcitabine chemotherapy.	BMC Cancer.	12:268		2012

IV. 研究成果の刊行物・別刷

Breast Cancer Res Treat (2013) 138:941–950
DOI 10.1007/s10549-013-2507-0

EPIDEMIOLOGY

Impact of recent parity on histopathological tumor features and breast cancer outcome in premenopausal Japanese women

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Abstract Although previous studies have reported that onset at young age is associated with poor prognosis in breast cancer, the correlation between reproductive factors, breast cancer characteristics, and prognosis remains unclear. Five hundred and twenty-six premenopausal young women diagnosed with primary invasive breast cancer between January 2000 and December 2007 were included in this study. Patients were classified into four groups according to their reproductive history: women who gave birth within the previous 2 years (group A), women who gave birth between 3 and 5 years previously (group B), women who gave birth more than 5 years previously (group C), and nulliparous women (group N). The correlation between the time since last childbirth to diagnosis, histopathological tumor features, and breast cancer prognosis was evaluated. Breast cancer patients who had given birth more recently had more advanced stage tumors; larger sized tumors; a higher rate of axillary lymph node metastases; a higher histological tumor grade; and increased progesterone receptor (PgR)−, HER2+, and triple negative tumors than patients who had given birth less recently or not at all. Group A patients had significantly shorter survival times than patients in both groups C and N (log rank test; $p < 0.001$). After adjusting for tumor characteristics, the hazard ratio for death in group A was 2.19 compared with group N ($p = 0.036$), and the adjusted hazard ratio restricted to patients in group A with hormone-receptor-positive, and HER2− tumors was 3.07 ($p = 0.011$). Young breast cancer patients who had given birth more recently had tumors with more aggressive features and worse prognoses compared with patients who had given birth less recently or were nulliparous.

Keywords Reproductive history · Subtype · Prognosis · Breast cancer in young women

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Introduction

Many studies have reported that young breast cancer patients have a poor prognosis [1–4]; however, the value of age as a prognostic factor remains a matter of debate [5]. Epidemiological studies have suggested that endogenous host environments, such as reproductive history, body-mass index, and BRCA germline mutation, may correlate with breast cancer features and prognosis [6–14]. In addition, molecular subtypes are known to be associated with survival [15–17], although the correlation between host environments, including reproductive factors and molecular subtype, remains unclear. Our objective was to explore the impact of host-related factors on the histopathological tumor features and prognosis in breast cancer patients.

Patients and methods

Patients

All premenopausal women of 20–44 years of age diagnosed with primary invasive breast cancer between January 2000 and December 2007 at the National Cancer Center Hospital in Tokyo (526 patients) were included in the present study. Clinical and pathological information was retrieved from medical charts. The follow-up period was completed in December 2011, and the median duration of follow-up was 6.3 years (range: 0.1–11.7 years), during which time 90 patients died. This study protocol was approved by the institutional review board at the National Cancer Center Hospital in Tokyo.

Data collection

Data was collected from various sources, including clinical pathology reports and the patients themselves. A questionnaire was routinely used to assess baseline characteristics at the initial visit for all patients. It included host-related factors, such as body-mass index, smoking history, drinking habits, and family history of breast and/or ovarian cancer in first or second-degree relatives (FH), and menstrual and reproductive factors, such as age at menarche, number of pregnancies, number of children, age at first and last delivery, and duration of breastfeeding. Patients were classified into four groups according to their reproductive history: women who gave birth within the previous 2 years (group A), women who gave birth between 3 and 5 years previously (group B), women who gave birth more than 5 years previously (group C), and nulliparous women (group N). Tumor characteristics, including histopathology; estrogen receptor (ER), progesterone receptor (PgR), and human EGFR-related 2 (HER2) statuses; and histological grade were abstracted from the relevant diagnostic pathology reports. Clinical stage was determined according to the TNM clinical classification from the American Joint Committee on Cancer/The International Union Against Cancer (AJCC/UICC) 6th edition.

Breast cancer subtypes were categorized according to expression of ER, PgR, and HER2 determined by immunohistochemistry. Hormone-receptor positivity was defined as positive staining in more than 1 % of the tumor cell nuclei. HER2 positivity was defined as an immunohistochemistry score of 3+ (intense staining of the cell membrane in more than 30 % of the cancer cells) or an IHC score of 2+ and positive fluorescence in situ hybridization (FISH) HER2 amplification signals. Subtypes were defined as follows: HR+HER2−, ER− or PgR+, and HER2−; HR+HER2+, ER− or PgR+, and HER2+; HR−HER2−, ER, PgR−, and HER2− (triple negative); and HR−HER2+, ER− and PgR−, and HER2+ (HER2-enriched).

Statistical analyses

All statistical analyses were performed using SAS Ver. 9.2 statistical software (SAS Statistic Inc., Cary, NC). All the tests were two-sided, and p values of <0.05 were considered significant. For comparison of patient groups, the Chi squared test was used for discrete data, and the Wilcoxon rank sum test was used for continuous data. Overall survival (OS) was calculated from the first day of breast cancer diagnosis until death from any cause. Survival curves were derived from the Kaplan–Meier product limit estimate method, with the log-rank statistic being used to test for differences between groups. Hazard ratios and 95 % confidence intervals (CI) for death were estimated using Cox proportional hazards survival models, with and without adjusting for one or more of the following factors: age at diagnosis, AJCC stage, hormone receptor and HER2 statuses, and histological tumor grade. To determine any trends between age at diagnosis and time from last childbirth to diagnosis, linear regression was used for continuous data, whereas correlation and ANOVA statistics were used for discrete data.

Results

Patient and tumor characteristics

Clinical characteristics at diagnosis according to each group are presented in Table 1. The median age at diagnosis for all patients was 39 years (range: 22–44 years). No difference in the FH of breast cancer was observed between nulliparous and parous women. Among the 526 women included in this study, 37 women (7 %) were classified into

Table 1 Patient characteristics

	Parous			Nulliparous
Time since last parity: Number or patients:	Group A ≤2 years *N* = 37	Group B 3–5 years *N* = 59	Group C >5 years *N* = 181	Group N Nulliparous *N* = 249
Age at diagnosis, median (range)	35 (26–44)	37 (27–43)	41 (32–44)	38 (22–44)
Age at diagnosis category, *N* (%)				
<35	18 (49)	15 (25)	4 (2)	75 (30)
35–39	15 (41)	26 (44)	44 (24)	69 (28)
40–44	4 (11)	18 (31)	133 (73)	105 (42)
Family history of breast and/or ovarian cancer (within second degree), *N* (%)				
Absent	27 (73)	46 (78)	141 (78)	194 (78)
Present	10 (27)	13 (22)	40 (22)	55 (22)
Age at menarche, median (range)	12 (10–15)	12 (10–15)	12 (9–16)	12 (9–16)
Age at first full-term birth, median (range)	30 (23–43)	30 (20–38)	27 (19–38)	
Age at first full-term birth, category, N (%)				
Nulliparous				249 (100)
<30	17 (46)	26 (44)	137 (76)	
≥30	20 (54)	33 (56)	44 (24)	
Number of children, *N* (%)				
0 (nulliparous)				249 (100)
1	19 (51)	21 (36)	52 (29)	
2	11 (30)	29 (49)	105 (58)	
≥3	7 (19)	9 (15)	24 (13)	
Breastfeeding, *N* (%)				
Nulliparous				249 (100)
<6 months	15 (41)	22 (37)	60 (33)	
≥6 months	19 (51)	39 (66)	86 (48)	
Missing data	3 (8)	7 (12)	35 (19)	

group A, 59 (11 %) into group B, 118 (35 %) into group C, and 249 (47 %) into group N. Parous women with breast cancer were much older than nulliparous women, and the trend test showed that age at diagnosis increased as the period from last childbirth increased.

Tumor characteristics at diagnosis according to reproductive history are presented in Table 2. Between nulliparous and parous women, no significant differences were observed in any available factors. However, breast cancer patients who had given birth recently had more advanced stage tumors; larger sized tumors; a higher rate of axillary lymph node metastases; higher histological tumor grade; and more PgR−, HER2+, and triple negative tumors than those who had given birth less recently or not at all.

Impact of the time since last childbirth on outcome

The Kaplan–Meier 5-year OS probability was 64.3 % for group A, 79.3 % for group B, 88.2 % for group C, and 90.6 % for group N. The patients in group A had significantly shorter survival times than patients in both groups C and N (log rank test; $p < 0.001$ for both groups) (Fig. 1). Other host-related factors were not associated with survival.

Using multivariate Cox proportional hazards survival models, survival outcome of young breast cancer patients was associated with AJCC stage, histological tumor grade, and ER status, whereas age at diagnosis and PgR and HER2 statuses were not significantly associated with mortality. Using those models, breast cancer diagnosed within 2 years of last childbirth was an independently poor prognostic factor relative to nulliparity (Table 3). After adjusting for tumor characteristics, the hazard ratio for death in group A was 2.19 (95 % CI, 1.05–4.56; $p = 0.036$), 1.49 in group B (95 % CI, 0.79–2.83; $p = 0.223$), and 0.81 in group C (95 % CI, 0.46–1.43; $p = 0.471$) compared with group N (Table 4; Fig. 2). Among the patients with HR+HER2− tumors, the adjusted hazard ratio for death was 3.07 in group A (95 % CI, 1.30–7.27; $p = 0.011$), 1.01 in group B (95 % CI, 0.39–2.63; $p = 0.977$), and 0.60 in group C

Table 2 Tumor characteristics

Time since last parity:	Parous			Nulliparous	*p* value	
	Group A ≤2 years *N* = 37 *N* (%)	Group B 3–5 years *N* = 59 *N* (%)	Group C >5 years *N* = 181 *N* (%)	Group N nulliparous *N* = 249 *N* (%)	Parous vs. nulliparous	Trend test (parous)
AJCC stage at diagnosis					0.409	0.584
0	1 (3)	1 (2)	4 (2)	8 (3)		
I	5 (13)	16 (27)	52 (29)	60 (24)		
II	18 (49)	26 (44)	97 (53)	140 (56)		
III	9 (24)	14 (24)	21 (12)	34 (14)		
IV	4 (11)	2 (3)	7 (4)	7 (3)		
AJCC T factor at diagnosis					0.679	0.010
Tis	1 (3)	1 (2)	4 (2)	7 (3)		
T1	7 (19)	18 (30)	57 (31)	63 (25)		
T2	16 (43)	23 (39)	90 (50)	130 (52)		
T3	8 (22)	11 (19)	21 (12)	32 (13)		
T4	5 (13)	6 (10)	9 (5)	16 (6)		
T0 (Occult primary)	0 (0)	0 (0)	0 (0)	1 (0)		
Regional lymph node metastasis at diagnosis					0.153	0.005
Negative	19 (51)	37 (63)	133 (73)	184 (74)		
Positive	18 (49)	22 (37)	48 (27)	65 (26)		
Histological type					0.075	0.139
Invasive ductal carcinoma	35 (95)	49 (83)	164 (90)	226 (91)		
Invasive lobular carcinoma	0 (0)	2 (3)	10 (6)	3 (1)		
Others	2 (5)	8 (14)	7 (4)	20 (8)		
Estrogen receptor status					0.436	0.140
Negative	19 (51)	19 (32)	63 (35)	83 (33)		
Positive	18 (49)	39 (66)	117 (64)	165 (67)		
Missing data	0 (0)	1 (2)	1 (1)	1 (0)		
Progesterone receptor status					0.328	0.001
Negative	20 (54)	18 (30)	45 (25)	65 (26)		
Positive	17 (46)	40 (68)	135 (74)	182 (73)		
Missing data	0 (0)	1 (2)	1 (1)	2 (1)		
HER2 status					0.217	0.041
Negative	27 (73)	44 (74)	153 (84)	212 (85)		
Positive	10 (27)	14 (24)	27 (15)	36 (14)		
Missing	0 (0)	1 (2)	1 (1)	1 (0)		
Tumor subtype					0.605	0.004
HR+HER2−	16 (43)	38 (64)	128 (71)	174 (70)		
HR+HER2+	3 (8)	5 (9)	16 (9)	19 (8)		
HR−HER2− (TNBC)	11 (30)	6 (10)	25 (14)	38 (15)		
HR−HER2+	7 (19)	9 (15)	11 (6)	17 (7)		
Missing	0 (0)	1 (2)	1 (1)	1 (0)		
Histological tumor grade					0.253	0.005
Grade 1 and 2	9 (24)	27 (46)	95 (52)	131 (53)		
Grade 3	27 (73)	30 (51)	86 (48)	117 (47)		
Missing data	1 (3)	2 (3)	0 (0)	1 (0)		

AJCC American Joint Committee on Cancer, *HER2* human EGFR-related 2, *HR* hormone receptor, *TNBC* triple negative breast cancer

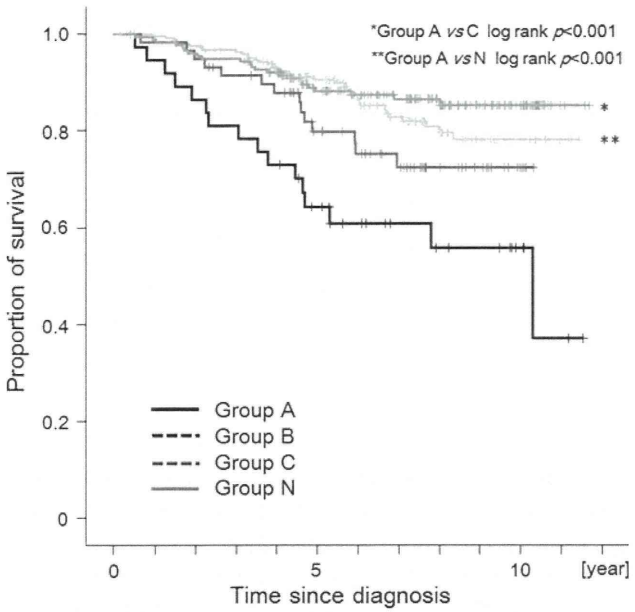

Fig. 1 Kaplan–Meier curves for overall survival based on the time since last childbirth

(95 % CI, 0.26–1.38; $p = 0.228$) compared to group N (Fig. 3a). However, among the patients with other tumor subtypes, no significant differences in survival were observed in any group (Fig. 3b–d). Other multivariate Cox proportional hazard survival models using age at first and last birth, time from first childbirth to diagnosis, or number of children among parous women were not associated with mortality (data not shown).

Discussion

Here, we showed that breast cancer patients with recent parity had shorter survival times than nulliparous patients. Women who had delivered within 2 years of breast cancer diagnosis had tumor(s) at a higher AJCC stage at diagnosis, a lower rate of ER− and PgR+ tumors, a higher rate of HER2+ and triple negative tumors, and a higher histological tumor grade than those with less recent childbirth. Even after adjusting for these well-known prognostic factors, including AJCC stage, hormone receptor and HER2 statuses, and histological tumor grade, women who delivered within 2 years of breast cancer diagnosis had a two-fold increased risk of death (i.e., were twice as likely to die) compared with nulliparous women. Moreover, when the analysis was restricted to patients with HR+HER2− tumors, women with recent parity had an even higher risk of death. Several studies have shown that breast cancer patients with recent childbirth before diagnosis had worse survival outcomes than nulliparous patients or those with a less recent childbirth [18–22]. However, to date, few studies have analyzed the hazard ratio adjusting for not only reproductive factors, but also tumor characteristics [23–26]. This study analyzed the hazard ratio adjusting for both reproductive factors and tumor characteristics, including hormone receptor and HER2 statuses and histological tumor grade.

The patients who were diagnosed with breast cancer within 2 years of parity might have had a delay in diagnosis as a result of pregnancy or lactation or have delayed

Table 3 Multivariate Cox proportional hazards survival models based on the time since last childbirth among patients with breast cancer

Factors	Status	Hazard ratio	95 % CI	Wald *p* value	3 test *p* value
AJCC stage	Stage 0–1	1			<0.0001
	Stage 2	2.63	1.10–6.30	0.0303	
	Stage 3–4	10.48	4.30–25.55	<0.0001	
Histological grade	Grade 1–2	1			NA
	Grade 3	2.49	1.47–4.21	0.0007	
ER status	Negative	1			NA
	Positive	0.66	0.39–1.12	0.125	
PgR status	Negative	1			NA
	Positive	0.94	0.55–1.60	0.8155	
HER2 status	Negative	1			NA
	Positive	1.08	0.61–1.92	0.7836	
Since last childbirth	Group N	1			0.0695
	Group A	2.19	1.05–4.56	0.0364	
	Group B	1.49	0.79–2.83	0.2231	
	Group C	0.81	0.46–1.43	0.4711	

Adjusted for age at diagnosis, AJCC stage, histological grade, and ER, PgR, and HER2 statuses

NA not applicable, *AJCC* American Joint Committee on Cancer, *HER2* human EGFR-related 2

Table 4 Hazard ratio for death based on the time since last childbirth

Since last childbirth	Unadjusted		Adjusted 1		Adjusted 2	
	HR (95 % CI)	*p*	HR (95 % CI)	*p*	HR (95 % CI)	*p*
Group N	1		1		1	
Group A	3.25 (1.81–5.85)	<0.001	2.26 (1.11–4.59)	0.024	2.19 (1.05–4.56)	0.036
Group B	1.59 (0.86–2.94)	0.141	1.50 (0.79–2.85)	0.210	1.49 (0.79–2.83)	0.223
Group C	0.79 (0.47–1.33)	0.377	0.81 (0.46–1.42)	0.460	0.81 (0.46–1.43)	0.471

Adjusted 1 HR adjusted for AJCC clinical stage (0–1, 2, 3–4), histological tumor grade (1–2, 3), and estrogen receptor status (positive, negative)

Adjusted 2 HR adjusted for age at diagnosis, AJCC clinical stage (0–1, 2, 3–4), histological tumor grade (1–2, 3), estrogen and progesterone receptor status (positive, negative), and HER2 status (positive and negative)

HR hazard ratio, *CI* confidence interval

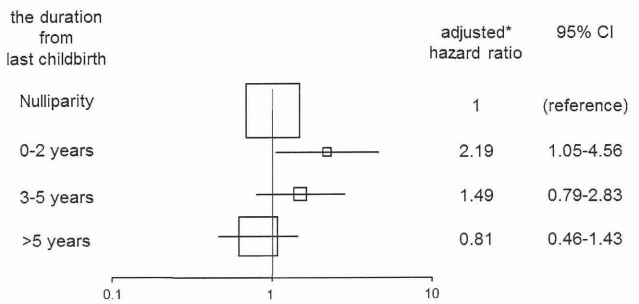

Fig. 2 Multivariate model of mortality based on the time since last childbirth. *Adjusted for age at diagnosis; AJCC clinical stage; histological tumor grade; and ER, PgR, and HER2 statuses; *CI* confidence interval

initiation of therapy until after delivery. Several studies had described that these factors also might have played a role in having an adverse outcome compared with those who had delivered more than 2 years earlier or were nulliparous at diagnosis [18, 21, 23–25]. This study showed that breast cancer patients who delivered within 2 years at diagnosis had more advanced T stage, more regional lymph node metastasis, and higher histological tumor grade compared with those who delivered 3 years or more at diagnosis by trend test. However, the time since last childbirth demonstrated an independent prognostic factor adjusted to tumor characteristics in our study.

The present study was concordant with previous studies showing that breast cancer patients with recent parity tend to have more advanced stage tumors, hormone-receptor negativity, aggressive growth, and high tumor grade, suggesting that pregnancy could have influenced tumor biology [21, 23, 27, 28]. Young breast cancer patients, those included women with recent childbirth, also had more aggressive tumor characteristics, less luminal A tumor, and more TNBC tumor [5, 16, 29, 30]. The present study was also concordant with epidemiological studies showing that recent parity before breast cancer diagnosis is associated with a worse outcome in premenopausal women (generally younger than 45 years), with a peak in risk of death within 2 years after delivery [21–26, 31]. Tumors found in women who have given birth recently have been reported to present with more adverse characteristics compared with tumors in nulliparous women [23, 32]. However, our results revealed that among patients with HR+HER2− tumors, which generally have a good prognosis, women who had given birth recently had a poorer prognosis than nulliparous women, although the reason for recent parity being associated with poor survival has not yet been clearly elucidated.

Pregnancy has a dual effect on the risk of breast cancer. A full-term pregnancy protects against the development of breast cancer later in life because full-term pregnancy induces differentiation of the mammary gland during pregnancy, making it less susceptible to carcinogenic insults [33]. However, shortly after pregnancy the risk of breast cancer increases temporarily, with a peak in risk 5–7 years after delivery [34, 35]. This short-term increase in risk may be because of stimulation of normal mammary gland growth by pregnancy hormones as well as, already existing mammary tumor cells.

Several hypotheses have been proposed to explain the poor prognosis of young breast cancer patients who have recently given birth. Gestational hormones, which are estrogen, progesterone, and insulin-like growth factor, increase tumor cell proliferation [36–40]. Special hormonal environment of pregnancy may influence the biology of more aggressive tumor type. Russo et al. [33] proposed that pregnancy induced differentiation of the mammary progenitor stem cell 1 to stem cell 2, which is less vulnerable to transformation by carcinogenic insult than progenitor stem cell 1. Recently, several studies have shown that the first full-term pregnancy induces a specific genomic signature in breast epithelium [41–43]. In the premenopausal parous human breast, inflammation-associated genes were upregulated and expression of hormone receptor and HER2 was changed compared to the nulliparous human breast of

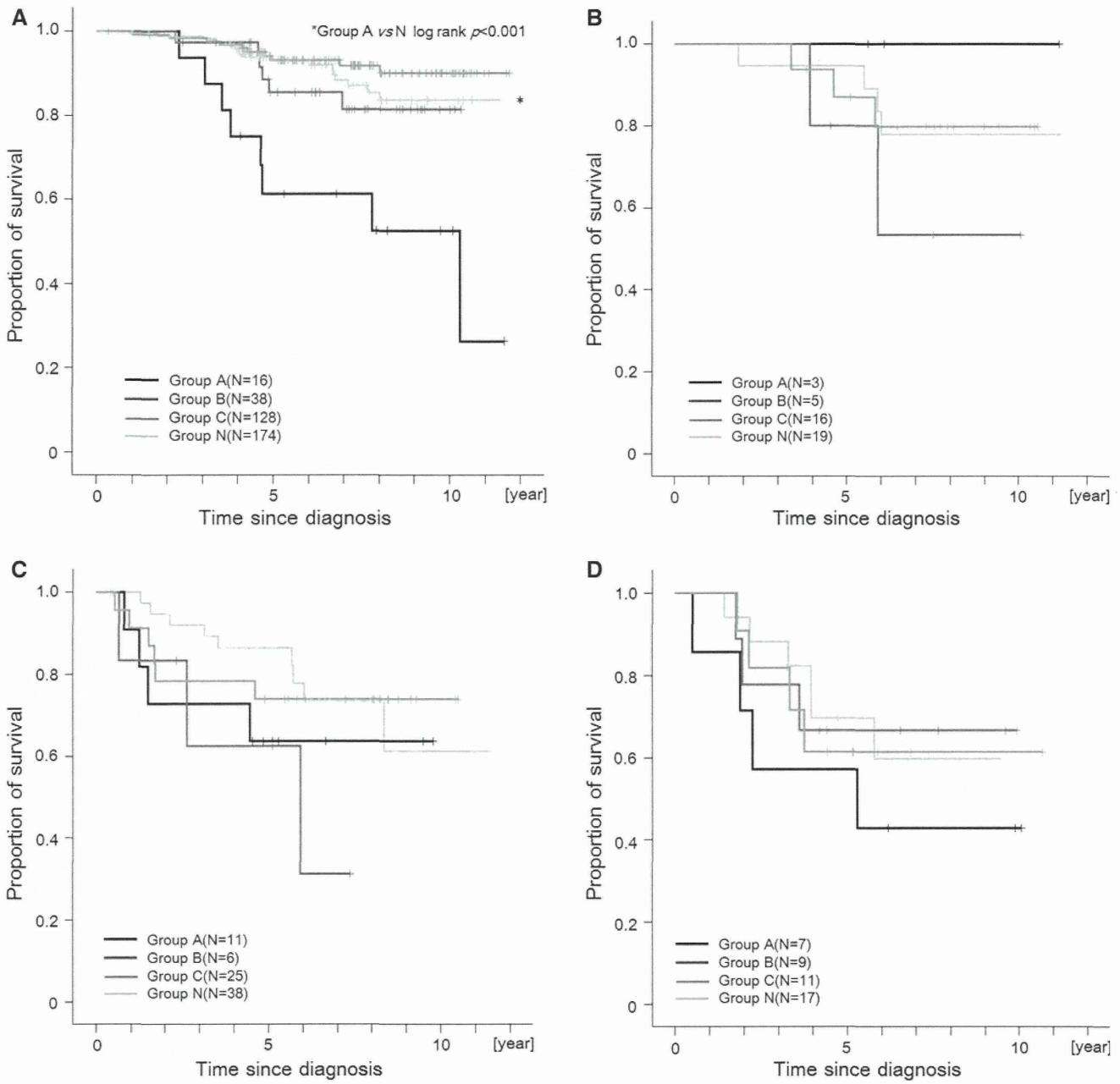

Fig. 3 Kaplan–Meier curves for overall survival according to tumor subtypes. **a** HR+HER2− subtype, **b** HR+HER2+ subtype, **c** HR−HER2− subtype, **d** HR−HER2+ subtype; *HR* hormone receptor

the same generation [42]. The genomic profile of the breast cancer cases, irrespective of parity history, differed from those of parous or nulliparous cancer-free cases according to the hierarchical clustering [41]. This finding suggests that the breast cancer cell was already generated before pregnancy and that pregnancy has contributed to prevention of mammary carcinogenesis. If a breast cancer cell had already been generated before the start of pregnancy, then estrogen and progesterone would mainly promote the proliferation of hormone-receptor-positive breast cancer cells, not negative cells.

This hypothesis cannot explain how a shorter length of time since the last childbirth leads to an increased development of hormone-receptor-negative breast cancer in young breast cancer patients. However, researchers have shown that receptor activator of nuclear factor-κB ligand secreted by progesterone-receptor-expressing epithelial cells stimulated by progesterone induced not only an epithelial proliferative response, but also epithelial carcinogenesis [44, 45]. In addition, RANKL PgR+ differentiated mammary cells stimulated by progesterone, promoted proliferation of the hormone-receptor-negative mammary

progenitor cells. Conversely, Schedin [35] proposed that the period between last childbirth to breast cancer diagnosis involved the process of mammary gland involution, which might facilitate breast cancer metastasis and increase the risk of death. In support of this hypothesis, others have shown that breast cancer patients with recent parity have a higher risk of distant recurrence than nulliparous women [46]. However, our data are not able to provide any proof for above-mentioned hypotheses underlying development of aggressive phenotype in women with recent parity.

Here, we have provided evidence that recent parity is associated with more aggressive histopathological tumor features and worse survival outcomes in breast cancer patients; however, our study does have some limitations. Firstly, since we used an initial routine questionnaire to assess reproductive status, some data was missing from our analysis. In fact, only 85 % of the data regarding breast-feeding status was obtained, although parity data from almost all patients was included in the analysis. Secondly, the questionnaire inquired information about prior use of any hormonal agents including those used for fertility treatment, contraception, and treatment for osteoporosis, but not all patients filled in the form and also their response had not been routinely validated through interview by healthcare providers. Thirdly, although the frequency of *BRCA1/2* germline mutation in Japanese women has been reported to be similar to caucasian in a small study [47], genetic counseling and testing has not been routinely recommended in clinical practice except for selected patients with a strong family history. Moreover *BRCA1/2* testing is not supported by public health insurance. Therefore, only a limited number of patients were offered genetic counseling and testing in this cohort, which disallows analyses according to *BRCA1/2* mutation status. However, family history was neither associated with clinical feature nor prognosis in our cohort (data not shown). Finally, it was not clear whether tumor(s) with poor outcome affected the advanced tumor characteristics or whether the advanced tumor characteristics caused the poor outcomes. However, our findings that breast cancer patients who gave birth more recently had poor outcomes even after adjusting well-known prognostic factors indicate that undiscovered factors associated with recent childbirth induce a change in the mammary glands. Further studies are needed to elucidate the underlying biology.

In conclusion, our results demonstrate that breast cancer patients who had given birth more recently had tumors with more aggressive features and a worse prognosis than patients who were nulliparous or had given birth less recently.

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Conflict of interest The authors declare that they have no conflict of interest.

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