MRPS23 amplification and gene expression in breast cancer; association with proliferation and the non-basal subtypes

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Abstract

Purpose MRPS23 is recognized as a driver of proliferation in luminal breast cancer. The aims of the present study were to describe MRPS23 copy number change in breast cancer, and to assess associations between MRPS23 copy number change and molecular subtype, proliferation and prognosis, and between MRPS23 gene expression and molecular subtype and prognosis.

Methods Using fluorescence in situ hybridization (FISH), we examined MRPS23 and centromere 17 copy number in 590 formalin-fixed, paraffin-embedded primary tumours and 144 corresponding lymph node metastases from a cohort of Norwegian breast cancer patients. Furthermore, we analysed MRPS23 gene expression data in 1971 primary breast cancer tumours from the METABRIC dataset. We used Pearson’s χ² test to assess associations between MRPS23 copy number and molecular subtype and proliferation, and between MRPS23 expression and molecular subtype. We studied prognosis by estimating hazard ratios and cumulative incidence of death from breast cancer according to MRPS23 copy number and MRPS23 expression status.

Results We found MRPS23 amplification (mean MRPS23 copy number ≥ 6 and/or MRPS23/chromosome 17 ratio ≥ 2) in 8% of primary tumours. Copy number increase associated with non-basal subtypes and higher tumour cell proliferation (Ki67). Higher MRPS23 expression associated with the Luminal B subtype. We found no significant association between MRPS23 amplification or MRSP23 gene expression, and prognosis.

Conclusion Amplification of MRPS23 is associated with higher proliferation and non-basal subtypes in breast cancer. High MRPS23 expression is associated with the Luminal B subtype.

Keywords MRPS23 · Breast cancer · Proliferation · Copy number · Amplification · METABRIC

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Introduction

Increased proliferation is a hallmark of cancer [1, 2], and identification of genetic drivers of proliferation could be important for prognostication and development of new targeted treatment. By high-throughput genomic analyses, Gatza et al. identified proliferation driving genes in non-basal breast cancer [3]. Amplification of four of these genes (MRPS23, FGDS5, DTX3 and METTL6) was associated with a poor prognosis. Mitochondrial ribosomal protein S23 (MRPS23) is located on the long arm of chromosome 17 (17q22) and belongs to the mitochondrial ribosomal protein gene family [4, 5]. Mitochondrial ribosomes are composed of a small 28S subunit and a large 39S subunit. MRPS23 encodes the 28S subunit [4, 5]. High MRPS23 expression has been found in colon [6], cervical [7, 8] and hepatocellular cancer [9, 10], and associated with poor prognosis in non-basal breast cancer [3], hepatocellular [10] and cervical cancer [7, 8]. In a breast cancer mouse model, MRPS23 knock-down reduced proliferation, induced apoptosis and limited angiogenesis and lymph node metastasis [11].

Our group has previously reclassified breast cancer tumours from a large cohort of Norwegian women into six molecular subtypes based on immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) [12]. The aims of the present study were to characterize MRPS23 copy number alterations by fluorescence in situ hybridization (FISH) on formalin-fixed, paraffin-embedded (FFPE) primary tumour tissue and corresponding lymph node metastases from this cohort, and to assess how these copy number alterations associate with molecular subtypes, proliferation and prognosis. Furthermore, using the METABRIC dataset [13], we assess how MRPS23 gene expression levels correlate with molecular subtypes and prognosis.

Materials and methods

Study populations and specimen characteristics

Cohort 1

Between 1956 and 1959, 25,727 women born 1886–1928 were invited to attend a clinical examination for early detection of breast cancer in Nord-Trøndelag County, Norway [14]. Through linkage with data from the Cancer Registry of Norway, these women were followed for breast cancer occurrence. Between 1961 and 2008, 1393 new breast cancers were registered. All tumours were reclassified according to histological type and grade [12, 15]. Tissue microarray (TMA) blocks were made using the Tissue Arrayer Mini-Core with TMA Designer2 software (Alphelys). Three 1-mm-in-diameter tissue cores from the periphery of the FFPE primary tumours and lymph node metastases were transferred to TMA recipient blocks. TMA sections (4 μm) were cut and stained, and the tumours were reclassified into molecular subtypes. Human epidermal growth factor receptor 2 (HER2) was assessed using both CISH and IHC. Tumours with HER2/ chromosome enumeration probe 17 (CEP17) ratio ≥ 2 were defined as HER2 positive. When CISH was unsuccessful, tumours with intense membranous staining (IHC 3+) in > 10% of tumour cells were considered HER2 positive.

Of the 1393 tumours, 909 were successfully reclassified into molecular subtypes [12]: Luminal A (ER and/or PR+, HER2−, Ki67 < 15%), Luminal B (HER2−) (ER+ and/or PR+, HER2−, Ki67 ≥ 15%), Luminal B (HER2+) (ER+ and/or PR+, HER2+), HER2 type (ER− and PR−, HER2+), 5 negative phenotype (5NP; ER−, PR−, HER2−, CK5− and EGFR−) and Basal phenotype (BP; ER−, PR−, HER2−, CK5− and EGFR+) (Supplementary Fig. 1). From the time of diagnosis (baseline), patients were followed until death from breast cancer, death from other causes or until December 31st, 2015. Individual information about adjuvant treatment is unavailable. However, due to age and/or time of diagnosis, few would have received chemotherapy. Some would have been treated with antihormonal treatment, but none qualified for trastuzumab. In the present study, TMA containing cores from tumours diagnosed mainly in the 1980s or later (n = 636) were included. Of these, 46 were excluded due to unsuccessful FISH (n = 30) or insufficient amounts of tumour tissue (n = 16). Thus, 590 cases were suitable for MRPS23 and CEP17 copy number assessment. Of these, 192 had lymph node metastases, and lymph node tissue from 150 was available in TMA. Due to unsuccessful FISH (n = 5) or insufficient amounts of tissue (n = 1), six were excluded. Hence, lymph node metastases from 144 cases were included.

Cohort 2 METABRIC

The METABRIC dataset includes a discovery dataset (n = 997), and a validation dataset (n = 995). The cohorts have been previously described in detail [13]. In the present study, 1971 cases had available follow-up data and MRPS23 gene expression data from all primary breast tumours. Tumours of basal-like (n = 329), normal-like (n = 202) and unknown subtype (n = 6) were excluded from our analysis [3, 16, 17]. Thus, 1434 tumours were included. Patients with ER positive and/or lymph node negative tumours had not received chemotherapy, whereas patients with ER negative and lymph node positive
tumours did [13]. None of the HER2+ patients were treated with trastuzumab. To assess possible associations between gene expression levels and prognosis, cases were separated into quartiles. Prognosis for each quartile was analysed separately, and for dichotomization of gene expression values, patients with gene expression levels in the upper quartile were compared to all other cases.

**MRPS23 FISH cohort 1**

FISH was done according to the manufacturer’s guidelines using Dako Histology FISH Accessory Kit K 579911. After de-waxing and rehydration, TMA slides were boiled in a microwave oven (10 min) in Pre-Treatment Solution, cooled (15 min) and washed in Wash Buffer (2 × 3 min). Protein digestion was performed with Pepsin Solution at 37 °C (30 min), and then washed in Wash buffer (2 × 3 min). Dehydration was done in ethanol (70, 80 and 95%) for 2 min at each concentration, and the slides were then air dried at room temperature for 15 min. FISH-custom probes for MRPS23 (3 μL, Empire Genomics) and CEP17 (1 μL, Abbott/VYSIS) were mixed with hybridization buffer (9 μL, Empire Genomics) and applied to TMA slides. Coverslips were applied and sealed with coverslip sealant (Dako). Denaturation was performed at 83 °C (3 min) followed by hybridization at 37 °C overnight in a DAKO Hybridizer. Post hybridization, TMA slides were rinsed in 0.4xSSC/0.3%NP-40 at 72 °C (2 min), and in 2xSSC/0.1%NP-40 at RT (15 s). Slides were air dried at 37 °C (15 min). DAPI (15 μL, VYSIS. Abbott no 06J50-001) was applied and the slides were coverslipped.

MRPS23 and CEP17 copy numbers were counted using a fluorescence microscope (Nikon Eclipse 90i). All available tissue cylinders from each case were examined, and MRPS23 and CEP17 copy number in 20 well-preserved, non-overlapping tumour cell nuclei were recorded. Mean copy number of MRPS23/tumour cell and MRPS23/CEP17 ratio were estimated for each case. To assess the impact of mean MRPS23 copy number, cases were divided into three categories based on recent HER2 guidelines [18]: mean MRPS23 copy number < 4; mean ≥ 4 < 6 and mean ≥ 6. To estimate the impact of gene/centromere ratio, cases were divided into two categories: MRPS23/CEP17 < 2 and MRPS23/CEP17 ≥ 2. Finally, MRPS23 amplification (MRPS23+) was defined as mean MRPS23 copy number ≥ 6 and/or MRPS23/CEP17 ratio ≥ 2. Cases with MRPS23 copy number < 6 and MRPS23/CEP17 ratio < 2 were defined as non-amplified (MRPS23−). To study the prognostic value of HER2 status and MRPS23 status combined, cases were divided into four groups: MRPS23+/HER2−; MRPS23+/HER2+; MRPS23+/HER2− and MRPS23+/HER2+. The REMARK criteria for tumour marker reporting were followed [19].

**Statistical analyses**

Pearson chi square tests were used to compare MRPS23 copy number status and MRPS23 gene expression levels in the primary tumours across patient and tumour characteristics. To compare copy number status in the primary tumours and their corresponding lymph node metastases, paired analyses were performed using McNemar’s and marginal homogeneity test.

For each category of MRPS23 copy number status and MRPS23/HER2 status in primary tumours, and for the MRPS23 gene expression categories, we estimated cumulative incidence of death from breast cancer, with death from other causes as a competing event. We used Gray’s test to compare equality of cumulative incidence curves. Cox proportional hazard models were used to estimate hazard ratios (HR) of breast cancer death with 95% confidence intervals (CI). In the Cox regression analyses, patients were censored at time of death from other causes. Where applicable, adjustments were made for age at baseline (≤ 49, 50–59, 60–64, 65–69, 70–74, ≥ 75), histological grade (I–III), stage (I–IV), Ki67 status (< / ≥ 15%) and molecular subtype. We found no clear violations of proportionality in log-minus-log plots. Linear regression analyses were used for comparison of MRPS23 expression levels between different molecular subtypes.

All statistical tests were two-sided, and statistical significance was assessed at 5% level. p-values between 5 and 10% were regarded borderline significant. All statistical analyses were done using STATA version 15.1 (Stata Corp., College Station, TX, USA).

**Results**

**Cohort 1**

Mean age at diagnosis was 75.6 (SD 8.6, range 41–96) years and mean follow-up after diagnosis was 8.9 (SD 7.2) years. By the end of follow-up, 217 (37%) patients had died from breast cancer, and 318 (54%) had died from other causes (Table 1).

**MRPS23 copy number status in primary tumours**

When MRPS23 and CEP17 copy number alterations were present, a homogenous pattern was seen, with alterations in the majority of tumour cells. Three different phenotypes were seen, tumours without copy number alterations; tumours with MRPS23 and CEP17 copy number increase and tumours with MRPS23 copy number increase only (Fig. 1). In total, 29 of the primary tumours (5%) had mean MRPS23 copy number ≥ 4 < 6, 21 (4%) had
Table 1 Characteristics of cohort 1

| Characteristic | Categories defined by mean MRPS23 | Categories defined by MRPS23/CEP17 ratio | Categories defined by amplification status |
|----------------|-----------------------------------|-----------------------------------------|--------------------------------------------|
| N (%)          | 590 (100)                         | 549 (93)                                | 545 (92)                                   |
| Mean age at diagnosis, years (SD) | 75.6 (8.6)                        | 75.6 (8.5)                              | 75.6 (8.5)                                |
| Deaths from BC (%) | 217 (37)                          | 199 (36)                               | 198 (36)                                  |
| Deaths from other causes (%) | 318 (54)                           | 297 (51)                               | 294 (54)                                  |
| Histologic grade (%) |                                  |                                        |                                            |
| I              | 70 (12)                           | 69 (13)                                | 69 (13)                                   |
| II             | 327 (55)                          | 308 (57)                               | 308 (56)                                  |
| III            | 193 (33)                          | 163 (30)                               | 168 (31)                                  |
| Lymph node metastasis (%) |                                  |                                        |                                            |
| Yes            | 192 (33)                          | 173 (32)                               | 175 (32)                                  |
| No             | 245 (41)                          | 226 (42)                               | 228 (42)                                  |
| Tumour size (%) |                                  |                                        |                                            |
| ≤2 cm          | 282 (48)                          | 262 (48)                               | 261 (48)                                  |
| >2 cm, ≤5 cm   | 96 (16)                           | 90 (16)                                | 89 (16)                                   |
| >5 cm          | 10 (2)                            | 10 (2)                                 | 10 (2)                                    |
| Stage (%)      |                                  |                                        |                                            |
| I              | 286 (49)                          | 269 (49)                               | 267 (49)                                  |
| II             | 239 (41)                          | 220 (40)                               | 219 (40)                                  |
| III            | 35 (6)                            | 33 (6)                                 | 33 (6)                                    |
| IV             | 28 (5)                            | 25 (5)                                 | 25 (5)                                    |
| Molecular subtype (%) |                                  |                                        |                                            |
| Luminal A      | 309 (52)                          | 301 (55)                               | 301 (55)                                  |
| Luminal B (HER2−) | 148 (25)                      | 132 (24)                               | 130 (24)                                  |
| Luminal B (HER2+) | 44 (7)                          | 31 (6)                                 | 31 (6)                                    |
| HER2 type      | 33 (6)                            | 29 (5)                                 | 27 (5)                                    |
| SNP            | 14 (2)                            | 14 (3)                                 | 14 (3)                                    |
| BP             | 42 (7)                            | 42 (8)                                 | 42 (8)                                    |
Table 1 (continued)

| Histological subtype (%) | Categories defined by mean MRPS23 | Categories defined by MRPS23/CEP17 ratio | Categories defined by amplification status | p value ($\chi^2$) |
|--------------------------|-----------------------------------|------------------------------------------|-------------------------------------------|------------------|
|                          | Total study population             | <4 ≥ 4 to <6 ≥ 6                         | MRPS23/CEP17 < 2 and mean MRPS23 < 6     | MRPS23/CEP17 ≥ 2 | p value ($\chi^2$) |
|                          |                                   | p value ($\chi^2$)                       | and/or mean MRPS23 ≥ 6                    |                  |
| Ductal carcinoma         | 409 (69)                          | 380 (69)                                 | 377 (69)                                  | 32 (71)          | 0.695               |
|                          | 373 (69)                          | 29 (71)                                  |                                           |                  |
|                          | 24 (83)                           |                                           |                                           |                  |
|                          | 12 (57)                           |                                           |                                           |                  |
|                          | < 6                               |                                           |                                           | 0.840            |
|                          | ≥ 6                               |                                           |                                           |                  |
| Lobular carcinoma        | 78 (13)                           | 74 (13)                                  | 74 (14)                                   | 4 (9)            |
|                          | 75 (14)                           | 4 (10)                                   |                                           |                  |
|                          | 0                                 |                                           |                                           |                  |
|                          | 3 (14)                            |                                           |                                           |                  |
| Tubular carcinoma        | 3 (1)                             | 3 (1)                                    | 3 (1)                                     | 0                |
|                          | 0                                 |                                           |                                           |                  |
|                          | 0                                 |                                           |                                           |                  |
| Mucinous carcinoma       | 30 (5)                            | 28 (5)                                   | 28 (5)                                    | 2 (4)            |
|                          | 28 (5)                            | 2 (5)                                    |                                           |                  |
|                          | 1 (3)                             | 1 (5)                                    |                                           |                  |
| Medullary carcinoma      | 14 (2)                            | 14 (3)                                   | 13 (2)                                    | 1 (2)            |
|                          | 12 (2)                            | 2 (5)                                    |                                           |                  |
|                          | 1 (3)                             | 1 (5)                                    |                                           |                  |
| Papillary carcinoma      | 27 (5)                            | 24 (4)                                   | 24 (2)                                    | 3 (7)            |
|                          | 23 (4)                            | 3 (7)                                    |                                           |                  |
|                          | 2 (7)                             | 1 (3)                                    |                                           |                  |
|                          | 10 (10)                           | 4 (10)                                   |                                           |                  |
| Metaplastic              | 8 (1)                             | 8 (1)                                    | 8 (1)                                     | 0                |
|                          | 8 (1)                             | 0                                        | 0                                         |                  |
|                          | 0                                 | 0                                        | 0                                         |                  |
| Other                    | 21 (4)                            | 18 (3)                                   | 18 (3)                                    | 3 (7)            |
|                          | 18 (3)                            | 3 (7)                                    |                                           |                  |
|                          | 2 (10)                            | 0                                        | 0                                         |                  |
| Ki67 high/low (%)        |                                    |                                         |                                           |                  |
| Ki67 < 15%               | 349 (59)                          | 335 (61)                                 | 334 (61)                                  | 15 (33)          | <0.001 |
|                          | 330 (61)                          | 14 (34)                                  |                                           |                  |
|                          | 6 (29)                            |                                           |                                           |                  |
| Ki67 ≥ 15%               | 241 (41)                          | 214 (39)                                 | 211 (39)                                  | 30 (66)          |
|                          | 210 (39)                          | 27 (66)                                  |                                           |                  |
|                          | 16 (55)                           |                                           |                                           |                  |
| Mitoses/10 HPF, median (IQR) | 5 (1.12)                         | 5 (1.12)                                 | 5 (1.12)                                  | 8 (4.17)         |
|                          | 5 (1.12)                          | 8 (3.16)                                 |                                           |                  |
|                          | 10 (5.16)                         |                                           |                                           |                  |
| Mitoses/10 HPF, quartiles (%) |                                    |                                         |                                           |                  |
| ≤ 1                      | 154 (26)                          | 148 (27)                                 | 148 (27)                                  | 6 (13)           | 0.058 |
| >1, ≥ 5                  | 158 (27)                          | 148 (27)                                 | 147 (27)                                  | 11 (24)          |
| >5, ≤ 12                 | 140 (24)                          | 129 (24)                                 | 129 (24)                                  | 11 (24)          |
| >12                      | 138 (23)                          | 124 (23)                                 | 121 (22)                                  | 17 (38)          |

N number of patients, SD standard deviation, BC breast cancer, HER2 Human epidermal growth factor receptor 2, 5NP five negative phenotype, BP basal phenotype, HPF High-power filed, IQR inter quartile range.
mean copy number ≥ 6 and 41 (7%) had MRPS23/CEP17 ratio ≥ 2 (Table 1, Fig. 2). A total of 45 tumours (8%) were amplified (mean MRPS23 ≥ 6 and/or MRPS23/CEP17 ≥ 2). Among cases with mean MRPS23 ≥ 6, four cases had MRPS23/CEP17 ratio < 2. Of cases with MRPS23/CEP17 ratio ≥ 2, 24 had mean MRPS23 < 6.

MRPS23 copy number increase (mean ≥ 4) was found within all molecular subtypes except the 5NP (Table 1). Amplifications were found in all molecular subtypes except the 5NP and the BP. Amplifications were seen in 30% of Luminal B (HER2+), 18% of HER2 type, 12% of Luminal B (HER2−), and 3% of Luminal A. Of the MRPS23 amplified tumours, 19 (42%) were HER2+, compared to 58 (11%) of non-amplified tumours.

Copy number status in lymph node metastases

In total, 144 cases were examined for MRPS23 copy number status in their lymph node metastases (Table 2). There were no significant changes in MRPS23 copy number status in the lymph node metastases compared to the corresponding primary tumours. Among the pairs of primary tumours and lymph node metastases, 14 (10%) primary tumours were classified as MRPS23+, and in 10 of these (71%), the corresponding lymph node metastases were also MRPS23+. MRPS23 amplification was also identified in the lymph node metastases of two MRPS23-tumours.

MRPS23 copy number status and proliferation

Of the MRPS23 amplified tumours, 66% had high Ki67 (≥ 15%), compared to 39% of the non-amplified tumours (Table 1). Mitotic counts were also higher in amplified tumours (borderline significance). Of the MRPS23 amplified tumours, 56% were grade III, compared to 31% of the non-amplified tumours. An association between MRPS23 copy number increase and high Ki67, and high histological grade was also found when MRPS23 copy number status was defined by MRPS23 mean and MRPS23/CEP17 ratio (Table 1). In the lymph node metastases, MRPS23 amplification was associated with high Ki67 (borderline significance, Table 3).

MRPS23 copy number status and prognosis

Mean MRPS23

The cumulative risk of death from breast cancer for cases with no copy number increase was 35% (95% CI 31–39) 10 years after diagnosis (Table 4; Fig. 3a). The corresponding risks for cases with mean copy number ≥ 4 < 6

![Fig. 1 Breast cancer cell nucleus with a two copies of MRPS23 and CEP17, b copy number increase of both MRPS23 and CEP17 and c copy number increase of MRPS23 without corresponding increase of CEP17](image)

![Fig. 2 Scatter plot of MRPS23 and CEP17 copy number in 590 breast cancer tumours](image)
and mean ≥ 6 was 34% (95% CI 20–55), and 43% (95% CI 25–66), respectively. In the Cox regression analysis, there were no clear differences in the rates of death between categories. Adjustments for age, stage, grade, Ki67 or HER2 status did not influence the results.

**MRPS23/CEP17 ratio**

After 10 years of follow-up, cases with MRPS23/CEP17 ratio < 2 had a cumulative risk of death from breast cancer of 30% (95% CI 27–34) (Table 4; Fig. 3b), whereas cases with ratio ≥ 2 had a corresponding risk of 41% (95% CI 30–58).

When comparing rates of death from breast cancer, there was no clear difference between the two categories (HR 1.2, 95% CI 0.8–2.0). Adjusting for age, stage, grade, Ki67 or HER2 status did not influence the results.

**MRPS23 amplification status**

After 10 years of follow-up, patients with MRPS23 amplified tumours had 40% (95% CI 27–56) cumulative risk of death from breast cancer, compared to 30% (95% CI 27–34) for patients without amplification (Table 4; Fig. 3c). The rates of death from breast cancer were similar for cases with and without amplification (HR 1.2, 95% CI 0.7–1.9). Separate adjustments for age, grade, histological grade, stage and HER2 status did not influence the results. Analysis of prognosis was also done for Luminal A cases separately, and for all luminal subtypes combined. In these analyses, no clear differences in prognosis between MRPS23 amplified and non-amplified cases were seen (data not shown).

**MRPS23/HER2− status**

When tumours were reclassified into four categories based on MRPS23 and HER2 status, the highest risk of death was

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Table 2  **MRPS23** status in primary tumours and lymph node metastases according to MRPS23/CEP17 ratio, mean MRPS23 and amplification status

| MRPS23/tumour cell, primary tumours | Mean MRPS23/tumour cell, primary tumours | Marginal homogeneity test |
|-------------------------------------|------------------------------------------|--------------------------|
| < 4 | ≥ 4, < 6 | ≥ 6 | Total |
| Mean MRPS23/tumour cell, lymph nodes | | | |
| < 4 | 125 (97) | 4 (40) | 1 (20) | 130 | $p = 0.637$ |
| ≥ 4, < 6 | 3 (2) | 5 (50) | 2 (40) | 10 |
| ≥ 6 | 1 (1) | 1 (10) | 2 (40) | 4 |
| Total | 129 | 10 | 5 | 144 |

| MRPS23/CEP17 ratio, primary tumours | MRPS23/CEP17 ratio, primary tumours | McNemar test |
|-------------------------------------|-------------------------------------|--------------|
| < 2 | ≥ 2 | Total |
| MRPS23/CEP17 ratio, lymph nodes | | | |
| < 2 | 130 (99) | 3 (23) | 133 | $p = 0.625$ |
| ≥ 2 | 1 (1) | 10 (76) | 11 |
| Total | 131 | 13 | 144 |

| Amplification status, primary tumours | Amplification status, primary tumours | McNemar test |
|-------------------------------------|-------------------------------------|--------------|
| MRPS23− | MRPS23+ | Total |
| Amplification status, lymph nodes | | | |
| $MRPS23^{−a}$ | 128 (98) | 4 (29) | 132 | $p = 0.688$ |
| $MRPS23^{+b}$ | 2 (2) | 10 (71) | 12 |
| Total | 130 | 14 | 144 |

*aMRPS23/CEP17 < 2 and mean MRPS23 < 6

bMRPS23/CEP17 ≥ 2 and/or mean MRPS23 ≥ 6

Table 3 **MRPS23** amplification status and Ki67 levels in lymph nodes

| MRPS23 amplification status, lymph node | MRPS23 amplification status, lymph node | $\chi^2$ | $p$ |
|-------------------------------------|-------------------------------------|----------|-----|
| $MRPS23^{−a}$ | $MRPS23^{+b}$ | Total |
| Ki67, lymph node (%) | | | |
| Ki67 < 15% | 69 (53%) | 3 (25%) | 72 | $p = 0.07$ |
| Ki67 > 15% | 62 (47%) | 9 (75%) | 71 |
| Total | 131 | 12 | 143 |

*aMRPS23/CEP17 < 2 and mean MRPS23 < 6

bMRPS23/CEP17 ≥ 2 and/or mean MRPS23 ≥ 6

and mean ≥ 6 was 34% (95% CI 20–55), and 43% (95% CI 25–66), respectively. In the Cox regression analysis, there were no clear differences in the rates of death between categories. Adjustments for age, stage, grade, Ki67 or HER2 status did not influence the results.

Table 3 MRPS23 amplification status and Ki67 levels in lymph nodes

| MRPS23 amplification status, lymph node | MRPS23 amplification status, lymph node | $\chi^2$ | $p$ |
|-------------------------------------|-------------------------------------|----------|-----|
| $MRPS23^{−a}$ | $MRPS23^{+b}$ | Total |
| Ki67, lymph node (%) | | | |
| Ki67 < 15% | 69 (53%) | 3 (25%) | 72 | $p = 0.07$ |
| Ki67 > 15% | 62 (47%) | 9 (75%) | 71 |
| Total | 131 | 12 | 143 |

*aMRPS23/CEP17 < 2 and mean MRPS23 < 6

bMRPS23/CEP17 ≥ 2 and/or mean MRPS23 ≥ 6

and mean ≥ 6 was 34% (95% CI 20–55), and 43% (95% CI 25–66), respectively. In the Cox regression analysis, there were no clear differences in the rates of death between categories. Adjustments for age, stage, grade, Ki67 or HER2 status did not influence the results.
**Table 4** Absolute and relative risk of death from breast cancer according to mean MRPS23/tumour cell, MRPS23/CEP17 ratio and amplification status

| CI confidence interval, HR hazard ratio | Mean MRPS23/tumour cell, primary tumour | MRPS23/CEP17 ratio, primary tumour | Amplification status, primary tumours |
|----------------------------------------|----------------------------------------|-----------------------------------|-------------------------------------|
|                                        | <4                                             | ≥4, < 6                             | ≥ 6                                 |
| Cum. risk after 5 years (%) (95% CI)    | 21 (18–24)                                   | 24 (12–44)                         | 38 (21–62)                          | 21 (18–25)                                   | 29 (18–46)                          | 29 (18–44)                          |<sup>a</sup>|<sup>b</sup>|
| Cum. risk after 10 years (%) (95% CI)   | 35 (31–39)                                   | 34 (20–55)                         | 43 (25–66)                          | 30 (27–34)                                   | 41 (30–58)                          | 40 (27–56)                          |<sup>a</sup>|<sup>b</sup>|
| HR, unadjusted (95% CI)                | 1.0                                           | 0.9 (0.5–1.7)                      | 1.5 (0.8–2.8)                      | 1.0                                           | 2.0 (0.8–3.8)                      | 2.0 (0.7–3.9)                      |<sup>a</sup>|<sup>b</sup>|
| HR adjusted for age (95% CI)           | 1.0                                           | 0.9 (0.5–1.7)                      | 1.5 (0.8–2.8)                      | 1.0                                           | 1.3 (0.8–2.1)                      | 1.0 (0.7–1.9)                      |<sup>a</sup>|<sup>b</sup>|
| HR adjusted for stage (95% CI)         | 1.0                                           | 0.8 (0.4–1.5)                      | 1.2 (0.6–2.4)                      | 1.0                                           | 1.2 (0.7–1.9)                      | 1.0 (0.6–1.7)                      |<sup>a</sup>|<sup>b</sup>|
| HR adjusted for grade (95% CI)         | 1.0                                           | 0.7 (0.4–1.4)                      | 1.3 (0.7–2.4)                      | 1.0                                           | 1.1 (0.7–1.7)                      | 1.0 (0.6–1.6)                      |<sup>a</sup>|<sup>b</sup>|
| HR adjusted for Ki67 (95% CI)          | 1.0                                           | 0.7 (0.4–1.4)                      | 1.1 (0.6–2.2)                      | 1.0                                           | 1.0 (0.6–1.6)                      | 1.0 (0.6–1.6)                      |<sup>a</sup>|<sup>b</sup>|
| HR adjusted for HER2 (95% CI)          | 1.0                                           | 0.9 (0.5–1.6)                      | 1.2 (0.6–2.3)                      | 1.0                                           | 1.0 (0.6–1.7)                      | 1.0 (0.6–1.6)                      |<sup>a</sup>|<sup>b</sup>|

**Fig. 3** Cumulative incidence of death from breast cancer according to MRPS23 copy number status based on a mean MRPS23 copy number (p = 0.47), b MRPS23/CEP17 ratio (p = 0.29) and c MRPS23 amplification status (p = 0.39)
After 10 years of follow-up, patients with the \textit{MRPS23}\textsuperscript{+}/HER2\textsuperscript{+} subtype had a cumulative risk of death from breast cancer of 63% (95% CI 42–83). The corresponding risk for the \textit{MRPS23}\textsuperscript{−}/HER2\textsuperscript{+} subtype was 47% (95% CI 36–61). The lowest risk of death was found in the \textit{MRPS23}\textsuperscript{+}/HER2\textsuperscript{−} subtype (23%, 95% CI 11–44). There were no clear differences in the rate of death from breast cancer between the \textit{MRPS23}\textsuperscript{−}/HER2\textsuperscript{+} and \textit{MRPS23}\textsuperscript{+}/HER2\textsuperscript{+} subtypes (HR 1.3, 95% CI 0.7–2.6). Separate analysis of prognosis was also done for all luminal cases, with similar results as for all cases combined (data not shown).

### Cohort 2

**MRPS23 expression according to molecular subtypes**

In the METABRIC dataset, mean age at diagnosis was 61.1 (SD 12.4, range 22–96) years, and mean follow-up after diagnosis was 8.1 (SD 4.9) years. Characteristics of the study population are given in Table 6. By the end of follow-up, 506 (26%) patients had died from breast cancer, and 384 (20%) had died from other causes.

High \textit{MRPS23} expression levels were associated with the Luminal B subtype (Fig. 5).

After 10 years of follow-up, cases with low \textit{MRPS23} expression levels had a cumulative risk of death from breast cancer of 26% (95% CI 23–29%), compared to 24% (95% CI 20–30%) among cases with high \textit{MRPS23} expression (Cut-off upper quartile; Fig. 6, Table 7). Comparing the rates of death from breast cancer, there were no significant differences between cases with gene expression levels in the upper quartile compared to the rest (HR 0.9, 95% CI 0.7–1.2, Fig. 6, Table 7). Similar results were obtained when analysis of prognosis was done for each quartile separately. Adjustments for age and histological grade did not influence the results.

### Discussion

We identified \textit{MRPS23} amplification in 8% of primary tumours and 9% of lymph node metastases in a large population of Norwegian breast cancer patients. The highest proportion of amplified cases was found within Luminal B (HER2\textsuperscript{+}), HER2 type and Luminal B (HER2\textsuperscript{−}) tumours. None of the amplified tumours were triple negative (5NP/ BP). \textit{MRPS23} amplification was associated with high Ki67 and high histological grade. No clear association between

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**Table 5** Absolute and relative risk of dying from breast cancer according to HER2 status and amplification of \textit{MRPS23}

|                  | \textit{MRPS23}\textsuperscript{−}/HER2\textsuperscript{−} | \textit{MRPS23}\textsuperscript{−}/HER2\textsuperscript{+} | \textit{MRPS23}\textsuperscript{+}/HER2\textsuperscript{−} | \textit{MRPS23}\textsuperscript{+}/HER2\textsuperscript{+} |
|------------------|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| Cum. risk after 5 years (%) (95% CI) | 18 (15–22)                                               | 44 (32–58)                                               | 12 (4–32)                                               | 53 (33–76)                                               |
| Cum. risk after 10 years (%) (95% CI) | 28 (25–32)                                               | 47 (36–61)                                               | 23 (11–44)                                               | 63 (42–83)                                               |
| HR, unadjusted (95% CI) | 1.0                                                      | 1.6 (1.1–2.4)                                            | 0.6 (0.3–1.4)                                            | 2.6 (1.5–4.5)                                            |
| HR adjusted for age (95% CI) | 1.0                                                      | 1.7 (1.1–2.5)                                            | 0.6 (0.3–1.4)                                            | 2.6 (1.5–4.8)                                            |
| HR adjusted for grade (95% CI) | 1.0                                                      | 1.2 (0.8–1.8)                                            | 0.5 (0.2–1.1)                                            | 2.3 (1.3–4.0)                                            |
| HR adjusted for stage (95% CI) | 1.0                                                      | 1.9 (1.3–2.8)                                            | 0.6 (0.3–1.4)                                            | 1.8 (1.0–3.3)                                            |
| HR adjusted for Ki67 (95% CI) | 1.0                                                      | 1.3 (0.9–1.9)                                            | 0.5 (0.2–1.0)                                            | 2.2 (1.3–3.0)                                            |
| HR adjusted for HER2 (95% CI) | 1.0                                                      | 1.6 (1.1–2.4)                                            | 0.6 (0.3–1.4)                                            | 2.6 (1.5–4.5)                                            |

Cum. risk cumulative risk, CI confidence interval, HR hazard ratio

\textsuperscript{a}MPR23\textsuperscript{−}: MPRS23/CEP17 ratio < 2 and MPRS23 mean < 6

\textsuperscript{b}MRPS23\textsuperscript{+}: MPRS23/CEP17 ratio ≥ 2 and/or MPRS23 mean ≥ 6

\textsuperscript{c}HER2/CEP17 ratio ≥ 2 or intense membranous staining (IHC 3+) in > 10% of tumour cells
MRPS23 amplification and prognosis was seen. The proportion of HER2 positive cases was higher among MRPS23 amplified cases, compared to non-amplified. MRPS23+/HER2+ had the poorest prognosis.

In the METABRIC dataset, Luminal B tumours had the highest level of MRPS23 gene expression. We found no statistically significant associations between MRPS23 expression levels and prognosis.

This study is based on a well-described cohort of breast cancer patients with long-term follow-up, and data from the METABRIC dataset. In the Norwegian cohort, the majority of patients have been followed until death [12]. Since relapse may occur even decades after the primary diagnosis, long-term follow-up is of particular value in breast cancer research. Molecular subtyping was performed in the same laboratory, using the same algorithm and antibodies in all cases [12]. Using FISH, gene copy number can be assessed while observing the morphology of the tumour, ensuring that only invasive tumour cells were examined.

MRPS23 copy number in primary tumours and lymph node metastases was assessed in TMAs. In the primary tumours, tissue for TMAs was taken from the tumour periphery. Previous studies have shown good correlation between TMAs and corresponding whole sections [20, 21]. Nevertheless, TMAs represent a small portion of each tumour, and, while copy number changes were observed throughout the tissue in amplified cases, intra tumour heterogeneity may not

| N (%)         | Total | Mean probe MRPS23 | χ²          | Discovery cohort | Validation cohort |
|---------------|-------|-------------------|-------------|------------------|-------------------|
|               | N     | Quartile 1–3a     | Quartile 4b | Total            |                   |
| N (%)         | 1434  | 1069              | 365         | 804 (56)         | 630 (44)          |
| Mean age at diagnosis, years (SD) | 63.2 (12.4) | 63.6 (12.4) | 62 (12.4) | 62.3 (12.5) | 64.3 (12.2) |
| Mean follow-up, years (SD)       | 8.3 (4.9)  | 8.5 (5)          | 7.8 (4.5)   | 8.1 (4.7)   | 8.5 (5.1)      |
| Deaths from BC (%)              | 354 (25) | 273 (25)         | 81 (22)     | 201 (25)    | 153 (24)       |
| Deaths from other causes (%)     | 311 (22) | 240 (22)         | 71 (19)     | 156 (19)    | 155 (25)       |
| Histologic grade (%)            |        |                  | <0.001      | 61 (8)       | 78 (12)        |
| I                          | 139 (10)| 116 (11)         | 23 (6)      | 383 (48)    | 301 (48)       |
| II                         | 635 (44)| 504 (47)         | 131 (36)    | 421 (52)    | 329 (52)       |
| III                        | 598 (42)| 392 (17)         | 206 (56)    | 368 (46)    | 230 (37)       |
| Unknown                    | 62 (4)  | 57 (5)           | 5 (1)       | 0           | 62 (10)        |
| Lymph node metastasis (%)     |        |                  | 0.170       | 351 (42)    | 262 (42)       |
| Yes                       | 684 (48)| 491 (46)         | 193 (53)    | 421 (52)    | 330 (53)       |
| No                        | 750 (52)| 578 (54)         | 172 (47)    | 32 (4)      | 37 (6)         |
| Tumour size (%)             |        |                  | 0.075       | 104 (17)    | 177 (28)       |
| ≤2 cm                       | 613 (43)| 472 (44)         | 141 (39)    | 0           | 177 (28)       |
| >2 cm, ≤5 cm               | 751 (52)| 542 (51)         | 209 (57)    | 0           | 27 (4)         |
| >5 cm                      | 69 (5)  | 54 (5)           | 15 (4)      | 0           | 37 (6)         |
| Uncertain                   | 1 (0)   | 0                | 0           | 0           | 1 (0)          |
| Stage (%)                   |        |                  | 0.075       | 104 (17)    | 177 (28)       |
| I                          | 104 (7) | 84 (8)           | 20 (5)      | 0           | 104 (17)       |
| II                         | 177 (12)| 141 (13)        | 36 (10)     | 0           | 177 (28)       |
| III                        | 27 (2)  | 20 (2)           | 7 (2)       | 0           | 27 (4)         |
| IV                         | 1 (0)   | 0                | 0           | 0           | 1 (0)          |
| Unknown                    | 1125 (78)| 824 (77)        | 301 (82)    | 804 (100)  | 321 (50)       |
| PAM50 subtype (%)           |        |                  | 0.075       | 104 (17)    | 177 (28)       |
| Luminal A                  | 709 (49)| 606 (57)         | 103 (28)    | 445 (56)    | 255 (40)       |
| Luminal B                  | 488 (34)| 276 (26)         | 212 (58)    | 266 (33)    | 222 (35)       |
| HER2-type                  | 237 (17)| 187 (17)         | 50 (14)     | 84 (10)     | 153 (25)       |

Table 6 Characteristics of Cohort 2 (METABRIC) (Normal-like and basal-like subtypes excluded from the analyses)

N number of patients, SD standard deviation, BC breast cancer

aMean probe MRPS23 ≤ 8.31
bMean probe MRPS23 > 8.31
be captured. It would therefore be of interest to validate our findings in a study of whole sections.

There are no established guidelines as to how **MRPS23** amplification should be defined. According to **HER2** ISH guidelines, both **HER2** copy number and **HER2**/CEP17-ratio are taken into consideration [18]. **MRPS23** and **HER2** are both located on the long arm of chromosome 17 [4, 5, 22], and we chose to define **MRPS23** amplification according to **HER2** ISH guidelines, including both mean **MRPS23** copy number and **MRPS23**/CEP17 ratio in our definition. Previous studies have shown a high frequency of abnormalities on chromosome 17, but rarely true polysomy [23]. The number of amplified cases was increased when including **MRPS23**/CEP17 ratio in addition to mean **MRPS23** in the definition of amplification. Hence, our definition may have led to overestimation of **MRPS23** amplified cases. Nevertheless, we found that 8% of tumours were **MRPS23** amplified, whereas 20% and 33% of the tumours were amplified in the two datasets included in Gatza et al. In the latter two cohorts, only luminal (defined as non-basal) cases were included. When excluding the BP and 5NP in our in-house cohort, the proportion of amplified cases was still 8%. In accordance with other studies we found that amplification of **MRPS23** was associated with higher proliferation [3, 11]. However, contrary to others, we found no clear associations between **MRPS23** copy number increase and a poorer prognosis [3]. Our study demonstrates the importance of validating biomarkers identified by high-throughput genomic analyses. Validation analyses of single biomarkers with FISH, performed in FFPE tissue, indicate the marker’s prognostic potential when assessed in a routine diagnostic setting.

**MRPS23** has previously been found to be amplified exclusively in highly proliferative luminal tumours [3]. In that study, PAM50 was used for molecular subtyping, and “luminal” was defined as all tumours that were not basal [3]. This definition of luminal was based on a study showing that breast tumours could be separated into two main groups, one group containing luminal and **HER2**-positive tumours and the other group comprising basal-like tumours [17]. In our study, cohort 1 was divided into six subtypes based on IHC and ISH. Although it has been shown that surrogate markers can be used for molecular subtyping [24–27], there is a discrepancy between molecular subtype defined by surrogate markers and subtypes defined by gene expression analyses [28, 29]. Nevertheless, similar to Gatza et al., we only found **MRPS23** amplified cases among the non-basal tumours.

Contrary to others [3], we found no clear associations between **MRPS23** amplification and prognosis in our cohort of Norwegian breast cancer patients. We used a different method for assessment of gene copy number in

![Fig. 5](image1.png)  
**Fig. 5** **MRPS23** gene expression according to molecular subtype in 1971 patients from the METABRIC dataset

![Fig. 6](image2.png)  
**Fig. 6** Cumulative incidence of death from breast cancer according to **MRPS23** gene expression divided into a quartiles 1–4 (p=0.4), and b quartile 1–3 vs. quartile 4 (p=0.6)
our study (FISH), and different methodologies could partly explain the divergent results. Furthermore, the number of amplified cases was low in our study population, and the results must be interpreted with caution. Patient age in Cohort 1 is high, and the majority of patients were not given modern breast cancer treatment either due to their age at diagnosis or time of diagnosis [12]. This enables us to follow the near-natural course of disease after surgery. It would, however, be interesting to perform a MRPS23 FISH study in a cohort of younger patients treated according to current guidelines.

Interestingly, the MRPS23+/HER2+ tumours had the poorest prognosis. HER2 is recognized as an important prognostic marker in breast cancer, and interaction with MRPS23 could potentially be of clinical importance. However, due to the low number of cases in some of the MRPS23/HER2 categories, the results should be interpreted with caution.

We found no correlation between MRPS23 expression levels and prognosis in the METABRIC data set. In our analyses of prognosis, we excluded basal-like and normal-like cases [3, 16]. A correlation between MRPS23 copy number status and gene expression has previously been found in the METABRIC dataset [3]. Since transcription is regulated by several mechanisms, good correlation between gene copy number and gene expression is infrequent [30–32]. Our analyses show that MRPS23 expression levels were overlapping between the different molecular subtypes. Such overlap was also seen between the highly proliferative luminal tumours (Luminal B) and basal-like tumours, the latter shown to be non-amplified. This could possibly be due to other MRPS23 up-regulating mechanisms, and potentially explain the lack of correlation between gene expression levels and prognosis in the METABRIC dataset.

### Conclusion

Using FISH on a large cohort of breast cancer patients we found that MRPS23 amplification is associated with higher tumour cell proliferation. Amplifications were only found in luminal and HER2 type tumours. We found no association between MRPS23 amplification and prognosis. In the METABRIC dataset, gene expression levels were highest in Luminal B tumours. There was no correlation between MRPS23 expression and prognosis.

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### Compliance with ethical standards

**Conflict of interest** All authors declare that they have no conflicts of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved, including dispensation from the requirement

### Table 7 Absolute and relative risk of death from breast cancer according to gene expression levels of MRPS23, METABRIC data

| Mean probe MRPS23, quartiles | Mean probe MRPS23 |
|------------------------------|-------------------|
| Cum. risk after 5 years (%)  | 14 (11–18)        |
| (95% CI)                     | 14 (12–16)        |
| Cum. risk after 10 years (%) | 25 (20–30)        |
| (95% CI)                     | 26 (23–29)        |
| HR, unadjusted (95% CI)      | 1.0 (7.1–1.3)     |
| HR, adjusted for age (95% CI)| 1.0 (7.1–1.4)     |

* Cum. risk cumulative risk, CI confidence interval, HR hazard ratio; Luminal A, Luminal B and HER2-type included in analyses
  a Mean probe MRPS23 ≤ 7.80
  b Mean probe MRPS23 > 7.80, ≤ 8.05
  c Mean probe MRPS23 > 8.05, ≤ 8.31
  d Mean probe MRPS23 > 8.31
of patient consent, by the Regional committee for medical research ethics (REK 836-09).

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