

LETTERS

### High impact of methylation accumulation on metachronous gastric cancer: 5-year follow-up of a multicentre prospective cohort study

We recently published in your journal a 3-year multicentre prospective cohort study demonstrating the usefulness of an epigenetic cancer risk marker for gastric metachronous cancers.<sup>1</sup> This study achieved the first proof of concept of epigenetic cancer risk diagnosis in any type of cancer but, due to the short follow-up period, a relatively small number of events were observed, resulting in a marginally significant difference (p=0.042). It was anticipated that a longer follow-up could lead to a clearer difference and HR with a smaller 95% CI. We now report the 5-year follow-up data, which show highly significant results.

Among the 826 enrolled patients, 795 patients received annual follow-ups by endoscopy for a median period of 5.46 years (IQR: 3.95–6.09). By the end, 133 patients had developed a metachronous gastric cancer. Among them, 116 patients developed a metachronous gastric

cancer detected 1 year after the enrolment (authentic metachronous cancer).

Statistical analyses were conducted in the same manner as previously described.<sup>1</sup> Briefly, all the patients were categorised into quartiles (Q1: lowest to Q4: highest) according to the methylation levels of each of three genes (*miR-124a-3*, *EMX1* and *NKX6-1*). Cumulative incidences of metachronous gastric cancers were compared by a log-rank test, and HRs and 95% CIs were assessed by univariate and multivariate analyses by adjusting known risk factors and possible confounding factors, using a Cox proportional hazard regression model.

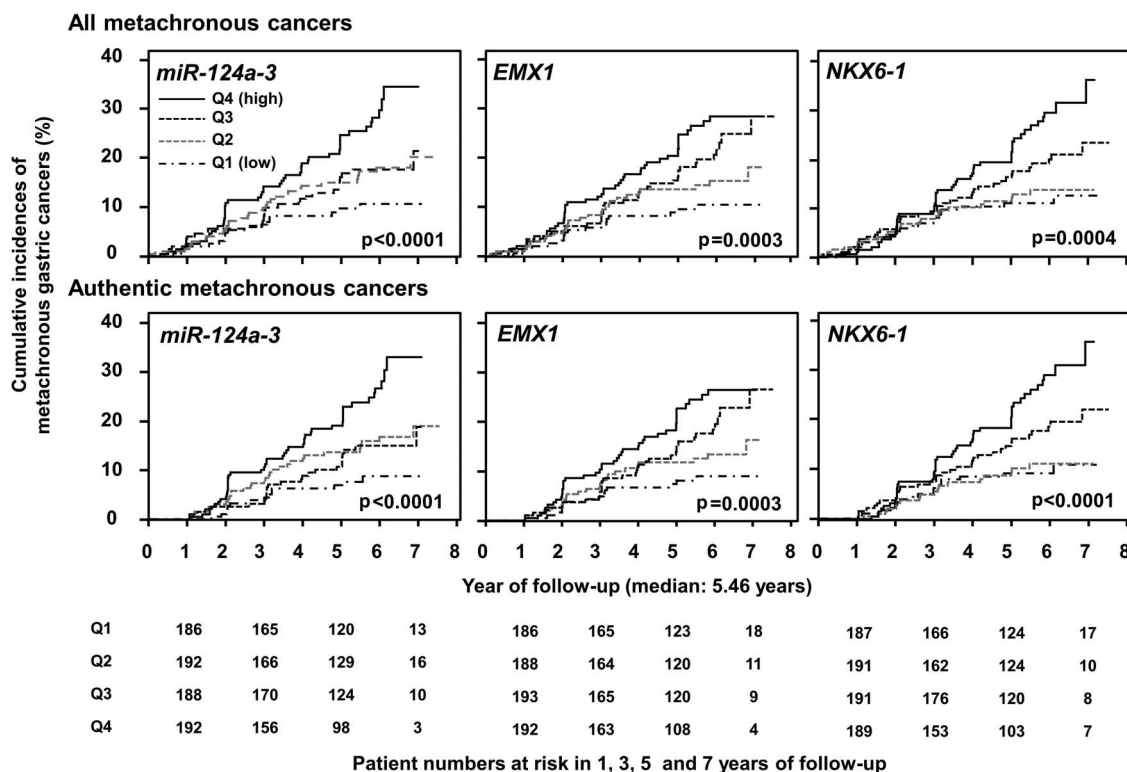
The univariate and multivariate analyses showed that Q4 (highest) of each of the three genes had significantly higher HRs than Q1 (lowest), using all and authentic metachronous gastric cancers (p<0.005) (table 1). Especially, the multivariate-adjusted HR of Q4 for *miR-124a-3* was 3.0 (95% CI 1.58 to 5.72, p=0.0017).

The Kaplan-Meier curves showed the cumulative incidences of the metachronous gastric cancers for quartiles (Q1–Q4) of methylation levels for each of the three genes (figure 1). For each gene, Q4 had a higher incidence of metachronous gastric cancer than Q1, with a p value of <0.001 by the log-rank test.

These final results based on the 5-year follow-up convincingly endorsed the proof of concept of epigenetic cancer risk diagnosis with sufficiently small p values, and provided a rationale that epigenetic markers can be used for cancer risk diagnosis. All the participants of this study once had a gastric cancer and thus originally carried a high risk of metachronous gastric cancer, as observed in Q1 (figure 1). Therefore, cancer risk stratification in this cohort was considered to be very difficult, but has been achieved. At the same time, the high risk inherent in the cohort will not allow changing the current clinical practice with annual endoscopic surveillance.

On the other hand, for asymptomatic *Helicobacter pylori*-infected individuals without a cancerous lesion, cancer risk stratification after their *H. pylori* eradication has been highly demanded because it can lead to optimisation of cancer surveillance based on an individual's risk. In order to establish precision medicine in this population, we have launched a new large-scale multicentre prospective cohort study (UMIN000016894) to predict the risk of primary gastric cancer in healthy individuals after *H. pylori* eradication.

The strong influence of methylation accumulation on gastric cancer risk was



**Figure 1** Cumulative incidences of metachronous gastric cancers of patients in quartiles (Q1–Q4) of methylation levels of *miR-124a-3*, *EMX1* and *NKX6-1*.

**Table 1** Univariate and multivariate-adjusted HRs (95% CI) for a metachronous gastric cancer according to DNA methylation levels of the three genes

|                                        |  | Univariate                        |                     |                     |                     |             |             | Multivariate*                     |                     |                     |             |  |  |
|----------------------------------------|--|-----------------------------------|---------------------|---------------------|---------------------|-------------|-------------|-----------------------------------|---------------------|---------------------|-------------|--|--|
|                                        |  | Quartile of DNA methylation level |                     |                     |                     |             |             | Quartile of DNA methylation level |                     |                     |             |  |  |
| Variable                               |  | Q1 (lowest)                       | Q2                  | Q3                  | Q4 (highest)        | p for trend | Q1 (lowest) | Q2                                | Q3                  | Q4 (highest)        | p for trend |  |  |
| No of patients (795)                   |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |
| <i>miR-124a-3</i>                      |  | 198                               | 199                 | 199                 | 199                 |             | 198         | 199                               | 199                 | 199                 |             |  |  |
| <i>EMX1</i>                            |  | 198                               | 199                 | 199                 | 199                 |             | 198         | 199                               | 199                 | 199                 |             |  |  |
| <i>NKX6-1</i>                          |  | 198                               | 199                 | 199                 | 199                 |             | 198         | 199                               | 199                 | 199                 |             |  |  |
| All metachronous gastric cancers       |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |
| <i>miR-124a-3</i>                      |  | 18                                | 33                  | 32                  | 50                  |             | 18          | 33                                | 32                  | 50                  |             |  |  |
| No of events                           |  | 1                                 | 1.73 (0.97 to 3.08) | 1.64 (0.91 to 2.94) | 2.89 (1.66 to 5.02) | 0.0002      | 1           | 1.64 (0.90 to 2.99)               | 1.48 (0.80 to 2.74) | 2.57 (1.43 to 4.61) | 0.002       |  |  |
| HR (95% CI)                            |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |
| <i>EMX1</i>                            |  | 18                                | 28                  | 40                  | 47                  |             | 18          | 28                                | 40                  | 47                  |             |  |  |
| No of events                           |  | 1                                 | 1.47 (0.81 to 2.67) | 2.09 (1.19 to 3.67) | 2.63 (1.51 to 4.56) | 0.0001      | 1           | 1.46 (0.79 to 2.68)               | 1.75 (0.98 to 3.13) | 2.33 (1.31 to 4.15) | 0.0027      |  |  |
| HR (95% CI)                            |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |
| <i>NKX6-1</i>                          |  | 21                                | 24                  | 37                  | 51                  |             | 21          | 24                                | 37                  | 51                  |             |  |  |
| No of events                           |  | 1                                 | 1.13 (0.63 to 2.05) | 1.65 (0.95 to 2.85) | 2.42 (1.42 to 4.10) | 0.0002      | 1           | 1.12 (0.61 to 2.06)               | 1.61 (0.91 to 2.84) | 2.26 (1.30 to 3.92) | 0.0008      |  |  |
| HR (95% CI)                            |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |
| Authentic metachronous gastric cancers |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |
| <i>miR-124a-3</i>                      |  | 14                                | 30                  | 26                  | 46                  |             | 14          | 30                                | 26                  | 46                  |             |  |  |
| No of events                           |  | 1                                 | 2.03 (1.07 to 3.85) | 1.73 (0.89 to 3.33) | 3.53 (1.91 to 6.53) | <0.0001     | 1           | 1.93 (1.00 to 3.75)               | 1.50 (0.75 to 2.97) | 3.00 (1.58 to 5.72) | 0.0017      |  |  |
| HR (95% CI)                            |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |
| <i>EMX1</i>                            |  | 15                                | 24                  | 35                  | 42                  |             | 15          | 24                                | 35                  | 42                  |             |  |  |
| No of events                           |  | 1                                 | 1.52 (0.79 to 2.90) | 2.21 (1.20 to 4.07) | 2.87 (1.58 to 5.23) | 0.0001      | 1           | 1.44 (0.74 to 2.81)               | 1.79 (0.95 to 3.38) | 2.45 (1.31 to 4.58) | 0.0028      |  |  |
| HR (95% CI)                            |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |
| <i>NKX6-1</i>                          |  | 17                                | 18                  | 33                  | 48                  |             | 17          | 18                                | 33                  | 48                  |             |  |  |
| No of events                           |  | 1                                 | 1.07 (0.55 to 2.09) | 1.86 (1.02 to 3.89) | 2.92 (1.64 to 5.20) | <0.0001     | 1           | 1.03 (0.52 to 2.04)               | 1.74 (0.93 to 3.24) | 2.63 (1.44 to 4.82) | 0.0001      |  |  |
| HR (95% CI)                            |  |                                   |                     |                     |                     |             |             |                                   |                     |                     |             |  |  |

\*Adjusted for hospital, gender and age (>50, 50-59, 60-69 or ≥70), pepsinogen index, history of endoscopic submucosal dissection (0, 1, 2 or 3 times), pack-years of smoking (0, 1-39 or ≥40) and green vegetable intake (≤2 days/week, 3-4 days/week or almost daily).

considered to be due to the major contribution of aberrant DNA methylation induced by *H. pylori* infection in gastric epithelial cells to gastric carcinogenesis, along with mutations produced by activation-induced cytidine deaminase.<sup>2</sup> The relatively small number of driver mutations after comprehensive mutation analyses<sup>3,4</sup> also supports the importance of methylation accumulation in gastric carcinogenesis.

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