Prognostic role of Wnt and Fzd gene families in acute myeloid leukaemia

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Abstract

Wnt-Fzd signalling pathway plays a critical role in acute myeloid leukaemia (AML) progression and oncogenicity. There is no study to investigate the prognostic value of Wnt and Fzd gene families in AML. Our study screened 84 AML patients receiving chemotherapy only and 71 also undergoing allogeneic haematopoietic stem cell transplantation (allo-HSCT) from the Cancer Genome Atlas (TCGA) database. We found that some Wnt and Fzd genes had significant positive correlations. The expression levels of Fzd gene family were independent of survival in AML patients. In the chemotherapy group, AML patients with high Wnt2B or Wnt11 expression had significantly shorter event-free survival (EFS) and overall survival (OS); high Wnt10A expressers had significantly longer OS than the low expressers (all \( P < .05 \)), whereas, in the allo-HSCT group, the expression levels of Wnt gene family were independent of survival. We further found that high expression of Wnt10A and Wnt11 had independent prognostic value, and the patients with high Wnt10A and low Wnt11 expression had the longest EFS and OS in the chemotherapy group. Pathway enrichment analysis showed that genes related to Wnt10A, Wnt11 and Wnt2B were mainly enriched in ‘cell morphogenesis involved in differentiation’, ‘haematopoietic cell lineage’, ‘platelet..."
1 | INTRODUCTION

The Wingless-Int (Wnt) gene family consists of 19 structurally related genes that encode secreted lipid-modified glycoproteins. These proteins initiate canonical and non-canonical signalling cascades through the transmission of Frizzled (Fzd) transmembrane receptors and lipoprotein-related protein (Lrp) single-transmembrane co-receptors. Wnt signalling pathway regulates a series of cellular processes such as proliferation, differentiation, migration and apoptosis, and maintains the balance between self-renewal and differentiation of stem cells involved in the pathogenesis of multiple types of human cancers. In haemopoietic tissues, Wnts not only play an essential role in the maturation of haemopoietic stem cells (HSCs) into mature blood cells, but also participate in myelopoiesis by regulating erythroid- and thrombopoiesis. Almost all members of the Wnt and Fzd gene families are expressed at least to some extent in different haematopoietic sites during development. Therefore, Wnt signalling activity is critical in the precise regulation of normal haemopoiesis, while abnormal expression of components of Wnt-Fzd signalling pathway can induce leukaemia.

Acute myeloid leukaemia (AML) is a highly heterogeneous disease and belongs to clonal tumours originating from HSCs or myeloid cell precursors. It is associated with excessive proliferation of progenitor cells and differentiation of cell-cycle arrest. The role of Wnt signalling in the pathogenesis of leukaemia has attracted much attention in recent years. Müller-Tidow et al first found that the activation of Wnt signalling is a common feature of several balanced translocations in AML. A study showed that poor survival of AML patients is associated with the hypermethylation of Wnt genes without changes in expression levels. Wnt5a, as a tumour suppressor in different haematopoietic sites during development. Wnt signalling activity is critical in the precise regulation of normal haemopoiesis, while abnormal expression of components of Wnt-Fzd signalling pathway can induce leukaemia.

Acute myeloid leukaemia (AML) is a highly heterogeneous disease and belongs to clonal tumours originating from HSCs or myeloid cell precursors. It is associated with excessive proliferation of progenitor cells and differentiation of cell-cycle arrest. The role of Wnt signalling in the pathogenesis of leukaemia has attracted much attention in recent years. Müller-Tidow et al first found that the activation of Wnt signalling is a common feature of several balanced translocations in AML. A study showed that poor survival of AML patients is associated with the hypermethylation of Wnt genes without changes in expression levels. Wnt5a, as a tumour suppressor in the Wnt gene family, exhibits decreased expression in AML cells. However, Wnt4 ligand regulates leukemic cell growth by blocking cells to G1 cell-cycle phase in an Fzd6-independent manner. The overexpression of Fzd4 receptor in AML makes it highly sensitive to the activity of Wnt ligands. In addition, Fzd3 expression is higher in AML patients compared with normal group. Therefore, the role of Wnt and Fzd gene families in the pathogenesis, development and prognosis of AML cannot be ignored.

Gene mutation and expression profiles are associated with AML progression and prognosis. Nucleophosmin 1 (NPM1) and fms-like tyrosine kinase 3-internal duplication (FLT3-ITD) mutations occur in 50% and 30% of AML patients, respectively, and have negative impact on AML prognosis. Our previous studies found that individual genes in the same gene family (FUT, DOK and PAK families) have different prognostic value in AML. To investigate the prognostic significance of Wnt-Fzd signalling pathway in AML, we analysed the effects of Wnt and Fzd gene families on survival of AML patients who either received chemotherapy alone or followed by allogeneic haematopoietic stem cell transplantation (allo-HSCT) and dug deep into the underlying mechanism.

2 | METHODS

2.1 | Patients

The study screened 84 AML patients who received chemotherapy only and 71 who also underwent allo-HSCT from the Cancer Genome Atlas (TCGA) database (https://cancergenomics.nih.gov/). We obtained the complete Wnt (Wnt1, Wnt2B, Wnt4, Wnt5B, Wnt6, Wnt8B, Wnt10A, Wnt11 and Wnt16) and Fzd (Fzd1 to Fzd9) expression data in peripheral blood of 155 AML patients at diagnosis. Next, plenty of clinical and molecular characteristics were collected, including age, gender, race, white blood cell (WBC) counts, bone marrow (BM) blasts, peripheral blood (PB) blasts, French-American-British (FAB) subtypes and the frequencies of known recurrent genetic mutations. We also collected follow-up data in these patients including event-free survival (EFS) and overall survival (OS) and defined them as the end points. EFS refers to the time from diagnosis to the first event including relapse and death or was censored at the last follow-up. OS refers to the time from diagnosis to death from any cause or the last follow-up. Another independent cohort (GSE12417) was used to verify the results of aforesaid cohort.

2.2 | Statistical analysis

Clinical characteristics of patients were displayed in descriptive statistics. The associations between Wnt and Fzd gene families were assessed by Spearman correlation analysis. The differences of two groups in numerical data and categorical data were calculated by
Mann-Whiney U test and Chi-square test, respectively. Univariate (Kaplan-Meier method and the log-rank test) and multivariate (Cox proportional hazard model) analyses were used to evaluate whether Wnt and Fzd gene expression could predict EFS and OS. Spearman correlation analysis was further used to screen gene expression profiles related to Wnt genes with prognostic significance. Then we used StringDB (https://string-db.org) for online protein-protein interaction (PPI) analysis, used Cytoscape to visualize the results and used CytoHubba for hub gene screening. Finally, we used Metascape (http://metascape.org/) to perform pathway enrichment analysis of gene expression profiles related to Wnt genes. A two-tailed \( P < 0.05 \) was defined as statistically significant. The SPSS 24.0 statistical software and R 3.5.0 software were used for statistical analyses, and the graphics were drawn by GraphPad Prism 7.0 software.

3 | RESULTS

3.1 | Association between Wnt and Fzd gene families in AML

Figure 1A shows that there were positive correlations between the expression of some genes from Wnt and Fzd families in AML patients undergoing chemotherapy only, and their correlation coefficients range from 0.215 (Wnt11 and Fzd7) to 0.449 (Wnt5B and Fzd5). Figure 1B shows that there were also positive correlations between the expression of some genes from Wnt and Fzd families in AML patients receiving allo-HSCT, and their correlation coefficients range from 0.239 (Wnt10A and Fzd8) to 0.531 (Wnt11 and Fzd7).

3.2 | Prognostic value of Wnt and Fzd gene families in AML

According to the median expression levels of Wnt and Fzd family members, AML patients in chemotherapy and allo-HSCT groups were divided into high and low expression subgroups. Table 1 and Table S1 present the differences of EFS and OS between high and low Wnt and Fzd gene expression subgroups. The expression levels of all Fzd genes were not associated with EFS and OS in both groups except that the expression of Fzd1 affected EFS in the allo-HSCT group. All Wnt genes were also independent of survival in the allo-HSCT group (all \( P > 0.05 \)). In the chemotherapy group, AML patients with high Wnt2B expression had significantly shorter EFS and OS compared with low Wnt2B expressers (all \( P < 0.05 \), Figure 2A,B); high Wnt11 expressers had significantly shorter EFS and OS than low expressers (all \( P < 0.001 \), Figure 2E,F), whereas high Wnt10A expression had favourable effect on OS (\( P = 0.043 \), Figure 2D).

The verified results showed that AML patients with high Fzd3 or Fzd5 expression had significantly longer OS; high expression of Wnt4, Wnt6 and Wnt7B also had positive effects on OS, while AML patients with high Wnt2B or Wnt16 expression had significantly shorter OS (all \( P < 0.05 \), Figure S1 and Figure S2).
Table 1: Comparison of EFS and OS between high and low expression of the Wnt gene family

| Variables                     | EFS          | OS          |
|-------------------------------|--------------|-------------|
|                               | χ²  | P-value | χ²  | P-value |
| Chemotherapy only group       |     |         |     |         |
| Wnt1(high vs. low)            | 3.162 | 0.075   | 2.913 | 0.088   |
| Wnt2B(high vs. low)           | 8.253 | 0.004   | 5.454 | 0.020   |
| Wnt4(high vs. low)            | 0.129 | 0.719   | 0.237 | 0.626   |
| Wnt5B(high vs. low)           | 0.709 | 0.400   | 0.552 | 0.458   |
| Wnt6(high vs. low)            | 2.277 | 0.131   | 1.722 | 0.189   |
| Wnt8B(high vs. low)           | 0.395 | 0.529   | 0.085 | 0.771   |
| Wnt10A(high vs. low)          | 3.754 | 0.053   | 4.089 | 0.043   |
| Wnt10B(high vs. low)          | 0.985 | 0.321   | 1.261 | 0.262   |
| Wnt11(high vs. low)           | 14.055 | <0.001 | 12.442 | <0.001 |
| Wnt16(high vs. low)           | 0.254 | 0.615   | 0.269 | 0.604   |
| Allo-HSCT group               |     |         |     |         |
| Wnt1(high vs. low)            | 1.181 | 0.277   | 1.726 | 0.189   |
| Wnt2B(high vs. low)           | 0.091 | 0.762   | 0.302 | 0.582   |
| Wnt4(high vs. low)            | 1.142 | 0.285   | 0.088 | 0.767   |
| Wnt5A(high vs. low)           | 0.214 | 0.643   | 2.165 | 0.141   |
| Wnt6(high vs. low)            | 1.156 | 0.282   | 2.260 | 0.133   |
| Wnt8B(high vs. low)           | 0.321 | 0.571   | 0.117 | 0.732   |
| Wnt10A(high vs. low)          | 0.100 | 0.752   | 0.046 | 0.830   |
| Wnt10B(high vs. low)          | 0.011 | 0.918   | 0.672 | 0.412   |
| Wnt11(high vs. low)           | 0.102 | 0.750   | 0.121 | 0.728   |
| Wnt16(high vs. low)           | 0.429 | 0.513   | 0.112 | 0.738   |

Abbreviations: Allo-HSCT, allogeneic haematopoietic stem cell transplantation; EFS, event-free survival; OS, overall survival.

3.3 | Association of Wnt2B/10A/11 expression with clinical and molecular characteristics in the chemotherapy group

As shown in Table 2, Wnt2B\textsuperscript{high} group had fewer AML-M4 patients than the Wnt2B\textsuperscript{low} group (P = .040). No significant differences were found in age, gender, race, WBC, BM blasts, PB blasts, other FAB subtypes, karyotype, risk stratification, relapse ratio and frequency of recurrent genetic mutations (FLT3, NPM1, DNMT3A, IDH1/IDH2, RUNX1, NRAS/KRAS, TET2, TP53 and MLL) between the Wnt2B\textsuperscript{low} and Wnt2B\textsuperscript{high} groups (all P > .05).

Wnt10A\textsuperscript{high} group had lower WBC (P = .006), BM blasts (P < .001) and PB blasts (P = .001) and more FAB-M4 patients (P = .040) than the Wnt10A\textsuperscript{low} group. Other clinical and molecular characteristics including age, gender, race, FAB subtypes (M0, M1, M2, M5, M6, and M7), karyotype, risk stratification, relapse ratio and frequency of recurrent genetic mutations (FLT3, NPM1, DNMT3A, IDH1/IDH2, RUNX1, NRAS/KRAS, TET2, TP53 and MLL) showed no significant differences between the two groups (all P > .05).

3.4 | Multivariate analysis of possible prognostic factors in the chemotherapy group

In order to further evaluate independent prognostic value of Wnt2B/10A/11 in patients treated with chemotherapy only, expression levels of Wnt2B/10A/11 (high vs. low), age (≥60 vs. <60 years), WBC (≥15 vs. <15 x 10\(^3\)/L), BM blasts (≥70 vs. <70%), PB blasts (≥20 vs. <20%), FLT3-ITD (positive vs. negative) and some genetic mutations (NPM1, DNMT3A, IDH1/IDH2, RUNX1, NRAS/KRAS, TET2, TP53 and MLL; mutated vs. wild) were included in multivariate analysis. As presented in Table 3, high Wnt11 expression was an independent risk factor for EFS and OS, along with age ≥ 60, BM blasts ≥ 70% and mutations in DNMT3A, RUNX1, TP53 and MLL (all P < .05): high Wnt2B expression and WBC count ≥ 15x10\(^3\)/L were independent risk factors for EFS (all P < .05), whereas high Wnt10A expression was an independent favourable factor for EFS (HR = 0.0560, P = .038) and OS (HR = 0.514, P = .018).

3.5 | Joint prognostic effects of Wnt10A and Wnt11 on AML

On the basis of multivariate analysis, we analysed the combined effects of Wnt10A and Wnt11 on prognosis of AML patients receiving chemotherapy only (Figure 3). We found that patients with high Wnt10A and low Wnt11 expression had significantly longer EFS and OS than those in Wnt10A/Wnt11\textsuperscript{high} (EFS: P = .013, OS: P = .013) and Wnt10A\textsuperscript{low}/Wnt11\textsuperscript{low} (EFS: P < .001, OS: P < .013) subgroups. No significant differences of EFS and OS were noticed between the Wnt10A\textsuperscript{high}/Wnt11\textsuperscript{low} and Wnt10A/Wnt11\textsuperscript{low} subgroups.

3.6 | Potential mechanism of Wnt2B/10A/11 in AML

Our further analysis indicated that the expression of Wnt2B, Wnt10A and Wnt11 were significantly correlated with 2337 genes, 3366 genes and 2147 genes, respectively (Figure 4A). Among the intersections of three related gene groups, the number of genes related to both Wnt10A and Wnt11 was the largest (369 genes), and the proteins encoded by these genes had a complex interaction network. The interaction network of proteins encoded by hub genes
Pathway enrichment analysis showed that genes related to \textit{Wnt10A} and \textit{Wnt11} were mainly enriched in 'cell morphogenesis involved in differentiation', 'blood vessel development', 'haemostasis' and 'haematopoietic cell lineage' signalling pathways (Figure 4C); genes related to \textit{Wnt2B} and \textit{Wnt10A} were mainly enriched in 'positive regulation of secretion', 'regulation of leukocyte-mediated cytotoxicity', 'Golgi vesicle transport' and 'platelet activation, signalling and aggregation' signalling pathways (Figure 4D); genes related to \textit{Wnt2B} and \textit{Wnt10} were mainly enriched in 'NAD metabolic process', 'proteasome-mediated ubiquitin-dependent protein catabolic process', 'positive regulation of CD4-positive, alpha-beta T-cell activation' and 'mitochondrial RNA metabolic process' signalling pathways (Figure 4E).
| Characteristics                      | Wnt2B High (n = 42) | Wnt2B Low (n = 42) | P-value | Wnt10A High (n = 42) | Wnt10A Low (n = 42) | P-value | Wnt11 High (n = 42) | Wnt11 Low (n = 42) | P-value |
|--------------------------------------|---------------------|--------------------|---------|----------------------|---------------------|---------|---------------------|---------------------|---------|
| Age/years, median (range)            | 66.5 (34.0, 88.0)   | 66.5 (22.0, 82.0)  | 0.465   | 67.0 (35.0, 88.0)    | 65.0 (22.0, 82.0)  | 0.342   | 69.0 (34.0, 82.0)   | 64.0 (22.0, 88.0)  | 0.09    |
| Age group/n (%)                      |                     |                    |         |                      |                     |         |                     |                     |         |
| < 60 years                           | 13 (31.0)           | 13 (31.0)          | 1.000   | 12 (28.6)            | 14 (33.3)          | 0.637   | 10 (23.8)           | 16 (38.1)          | 0.157   |
| ≥ 60 years                           | 29 (69.0)           | 29 (69.0)          | 0.827   | 30 (71.4)            | 28 (66.7)          |         | 32 (76.2)           | 26 (61.9)          |         |
| Gender/n (%)                         |                     |                    |         |                      |                     |         |                     |                     |         |
| Male                                 | 22 (52.4)           | 23 (54.8)          | 0.637   | 20 (47.6)            | 25 (59.5)          | 0.274   | 25 (59.5)           | 20 (47.6)          | 0.274   |
| Female                               | 20 (47.6)           | 19 (45.2)          | 0.274   | 22 (52.4)            | 17 (40.5)          |         | 17 (40.5)           | 22 (52.4)          |         |
| Race/n (%)                           |                     |                    |         |                      |                     |         |                     |                     |         |
| Caucasian                            | 28 (66.7)           | 35 (83.3)          | 0.078   | 33 (78.6)            | 30 (71.4)          | 0.450   | 30 (71.4)           | 33 (78.6)          | 0.450   |
| Others                               | 14 (33.3)           | 7 (16.7)           | 0.006   | 9 (21.4)             | 12 (28.6)          |         | 12 (28.6)           | 9 (21.4)           |         |
| WBC/×10^9/L, median (range)          | 14.4 (1.7, 297.4)   | 15.1 (0.7, 116.2)  | 0.181   | 8.6 (0.7, 131.5)     | 41.6 (1.0, 297.4)  | 0.006   | 13.3 (1.4, 297.4)   | 16.1 (0.7, 131.5)  | 0.993   |
| BM blasts/%, median (range)          | 73.5 (32.0, 99.0)   | 70.0 (30.0, 98.0)  | 0.001   | 63.5 (30.0, 91.0)    | 80.5 (40.0, 99.0)  | <0.001  | 71.5 (32.0, 99.0)   | 72.0 (30.0, 95.0)  | 0.900   |
| PB blasts/%, median (range)          | 23.5 (0.0, 98.0)    | 23.5 (0.0, 97.0)   | 0.504   | 11.5 (0.0, 97.0)     | 51.5 (0.0, 98.0)   | 0.001   | 24.5 (0.0, 98.0)    | 22.0 (0.0, 97.0)   | 0.882   |
| FAB subtypes/n (%)                   |                     |                    |         |                      |                     |         |                     |                     |         |
| M0                                   | 5 (11.9)            | 2 (4.8)            | 0.433   | 1 (2.4)              | 6 (14.3)           | 0.109   | 4 (9.5)             | 3 (7.1)            | 1.000   |
| M1                                   | 10 (23.8)           | 10 (23.8)          | 1.000   | 8 (19.0)             | 12 (28.6)          | 0.306   | 10 (23.8)           | 10 (23.8)          | 1.000   |
| M2                                   | 10 (23.8)           | 11 (26.2)          | 0.801   | 9 (21.4)             | 12 (28.6)          | 0.450   | 10 (23.8)           | 11 (26.2)          | 0.801   |
| M4                                   | 6 (14.3)            | 14 (33.3)          | 0.040   | 14 (33.3)            | 6 (14.3)           | 0.040   | 8 (19.0)            | 12 (28.6)          | 0.306   |
| M5                                   | 7 (16.7)            | 5 (11.9)           | 0.533   | 7 (16.7)             | 5 (11.9)           | 0.533   | 6 (14.3)            | 6 (14.3)           | 1.000   |
| M6                                   | 1 (2.4)             | 0 (0.0)            | 0.001   | 1 (2.4)              | 0 (0.0)            | 1.000   | 1 (2.4)             | 0 (0.0)            | 1.000   |
| M7                                   | 3 (7.1)             | 0 (0.0)            | 0.241   | 2 (4.8)              | 1 (2.4)            | 1.000   | 3 (7.1)             | 0 (0.0)            | 0.241   |
| Cytogenetics/n (%)                   |                     |                    |         |                      |                     |         |                     |                     |         |
| Normal                               | 19 (45.2)           | 21 (50.0)          | 0.662   | 21 (50.0)            | 19 (54.8)          | 0.662   | 16 (38.1)           | 24 (57.1)          | 0.081   |
| t(9;22)/BCR-ABL1                     | 1 (2.4)             | 0 (0.0)            | 0.001   | 1 (2.4)              | 0 (0.0)            | 1.000   | 1 (2.4)             | 0 (0.0)            | 1.000   |
| inv(16)/CBFβ-MYH11                   | 2 (4.8)             | 4 (9.5)            | 0.676   | 5 (11.9)             | 1 (2.4)            | 0.202   | 3 (7.1)             | 3 (7.1)            | 1.000   |
| Complex                              | 35 (83.3)           | 38 (90.5)          | 0.332   | 34 (81.0)            | 39 (92.9)          | 0.106   | 33 (78.6)           | 40 (95.2)          | 0.024   |
| 11q23/MLL                            | 2 (4.8)             | 1 (2.4)            | 0.001   | 1 (2.4)              | 2 (4.8)            | 1.000   | 1 (2.4)             | 2 (4.8)            | 1.000   |
| -7/-7q+                              | 3 (7.1)             | 0 (0.0)            | 0.241   | 1 (2.4)              | 2 (4.8)            | 1.000   | 3 (7.1)             | 0 (0.0)            | 0.241   |

(Continues)
| Characteristics          | Wnt2B |                  |                  | Wnt10A |                  |                  | Wnt11  |                  |                  |
|--------------------------|-------|------------------|------------------|--------|------------------|------------------|--------|------------------|------------------|
|                          |       | High (n = 42)    | Low (n = 42)     | P-value | High (n = 42)    | Low (n = 42)     | P-value | High (n = 42)    | Low (n = 42)     | P-value |
| t(8;21)/RUNX1-RUNX1T1    | 2 (4.8) | 4 (9.5)         | 0.676            |        | 1 (2.4)         | 5 (11.9)        | 0.202  | 2 (4.8)         | 4 (9.5)         | 0.676  |
| Others                   | 6 (14.3) | 8 (19.0)        | 0.558            |        | 5 (11.9)        | 9 (21.4)        | 0.242  | 7 (16.7)        | 7 (16.7)        | 1.000  |
| Risk/n (%)               |       |                  |                  |        |                  |                  |        |                  |                  |        |
| Good                     | 4 (9.5)  | 8 (19.0)        | 0.212            |        | 6 (14.3)        | 6 (14.3)        | 1.000  | 5 (11.9)        | 7 (16.7)        | 0.533  |
| Intermediate             | 22 (52.4) | 24 (57.1)      | 0.661            |        | 25 (59.5)        | 21 (50.0)       | 0.381  | 18 (42.9)        | 28 (66.7)       | 0.028  |
| Poor                     | 14 (33.3) | 9 (21.4)       | 0.221            |        | 11 (26.2)        | 12 (28.6)       | 0.807  | 16 (38.1)        | 7 (16.7)        | 0.028  |
| FLT3/n (%)               |       |                  |                  |        |                  |                  |        |                  |                  |        |
| FLT3-ITD                 | 10 (23.8) | 5 (11.9)       | 0.350            |        | 7 (16.7)        | 8 (19.0)        | 0.100  | 8 (19.1)        | 7 (16.7)        | 0.872  |
| FLT3-TKD                 | 3 (7.1)  | 4 (9.5)         | 1 (2.3)          |        | 1 (2.3)         | 6 (14.3)        |        | 4 (9.5)         | 3 (7.1)         |        |
| Wild type                | 29 (69.0) | 33 (78.6)      | 34 (81.0)        |        | 31 (73.8)       | 28 (66.7)       |        | 30 (71.4)       | 32 (76.2)       |        |
| NPM1/n (%)               |       |                  |                  |        |                  |                  |        |                  |                  |        |
| Mutation                 | 16 (38.1) | 11 (26.2)      | 0.243            |        | 11 (26.2)        | 16 (38.1)       | 0.243  | 13 (31.0)        | 14 (33.3)       | 0.815  |
| Wild type                | 26 (61.9) | 31 (73.8)      | 31 (73.8)        |        | 26 (61.9)       | 26 (61.9)       |        | 29 (69.0)       | 28 (66.7)       |        |
| DNMT3A/n (%)             |       |                  |                  |        |                  |                  |        |                  |                  |        |
| Mutation                 | 12 (28.6) | 11 (26.2)      | 0.807            |        | 10 (23.8)        | 13 (31.0)       | 0.463  | 11 (26.2)        | 12 (28.6)       | 0.807  |
| Wild type                | 30 (71.4) | 31 (73.8)      | 32 (76.2)        |        | 29 (69.0)       | 31 (73.8)       |        | 30 (71.4)       | 32 (76.2)       |        |
| IDH1/IDH2/n (%)          |       |                  |                  |        |                  |                  |        |                  |                  |        |
| Mutation                 | 5 (11.9)  | 10 (23.8)       | 0.154            |        | 7 (16.7)        | 8 (19.0)        | 0.776  | 4 (9.5)         | 11 (26.2)       | 0.046  |
| Wild type                | 37 (88.1) | 32 (76.2)      | 35 (83.3)        |        | 34 (81.0)       | 38 (90.5)       |        | 31 (73.8)       | 38 (90.5)       |        |
| RUNX1/n (%)              |       |                  |                  |        |                  |                  |        |                  |                  |        |
| Mutation                 | 7 (16.7)  | 7 (16.7)        | 1.000            |        | 9 (21.4)        | 5 (11.9)        | 0.242  | 6 (14.3)        | 6 (19.0)        | 0.558  |
| Wild type                | 35 (83.3) | 35 (83.3)      | 33 (78.6)        |        | 37 (88.1)       | 36 (85.7)       |        | 34 (81.0)       | 36 (85.7)       |        |
| NRAS/KRAS/n (%)          |       |                  |                  |        |                  |                  |        |                  |                  |        |
| Mutation                 | 5 (11.9)  | 7 (16.7)        | 0.533            |        | 7 (16.7)        | 5 (11.9)        | 0.533  | 7 (16.7)        | 5 (11.9)        | 0.533  |
| Wild type                | 37 (88.1) | 35 (83.3)      | 35 (83.3)        |        | 37 (88.1)       | 35 (83.3)       |        | 37 (88.1)       | 37 (88.1)       |        |
| TET2/n (%)               |       |                  |                  |        |                  |                  |        |                  |                  |        |
| Mutation                 | 4 (9.5)   | 7 (16.7)        | 0.332            |        | 6 (14.3)        | 5 (11.9)        | 0.746  | 6 (14.3)        | 5 (11.9)        | 0.746  |
| Wild type                | 38 (90.5) | 35 (83.3)      | 36 (85.7)        |        | 37 (88.1)       | 36 (85.7)       |        | 37 (88.1)       | 37 (88.1)       |        |
| TP53/n (%)               |       |                  |                  |        |                  |                  |        |                  |                  |        |
| Mutation                 | 7 (16.7)  | 4 (9.5)         | 0.332            |        | 8 (19.0)        | 3 (7.1)         | 0.106  | 9 (21.4)        | 2 (4.8)         | 0.024  |
| Wild type                | 35 (83.3) | 38 (90.5)      | 34 (81.0)        |        | 39 (92.9)       | 33 (78.6)       |        | 40 (95.2)       | 40 (95.2)       |        |
In this retrospective study, we found significant correlations between some members of Wnt and Fzd gene families. The Fzd gene family has no significant prognostic value in AML. High Wnt2B and Wnt11 expression indicates poor outcomes, while high Wnt10A expression indicates good outcomes in AML patients with chemotherapy alone, but they have no prognostic significance in AML patients receiving allo-HSCT.

Nineteen mammalian Wnt ligands can bind to ten Fzd receptors to activate different downstream pathways including Wnt/\( Ca^{2+}\), Wnt/\( \beta\)-catenin and Wnt/planar cell polarity.\(^{19}\) Therefore, the correlations between some forms of Wnt and Fzd are reasonable. Down-regulation of some miRNAs (miRNA-204, miRNA-142-3p, miRNA-27b) leads to the up-regulation of Fzds in endometrial cancer, cervical cancer and gastric tumour.\(^{20-22}\) In AML, the knockdown of miRNA-126 increases the expression of Fzd7, leading to aggravation of the disease.\(^{23}\) However, we observed that the expression of Fzds has no significant effect on the survival of AML patients, which may be because the expression of Fzds can be indirectly affected by some miRNAs. The activation of Wnt signalling induces the expression of ETO and AML1 and increases the possibility of their translocation.\(^{24}\)

AML1-ETO fusion protein can positively regulate the transcription of plakoglobin, which plays an important role in enhancing the self-renewal of HSCs.\(^{25}\) The expression of Wnt2B, Wnt10A and Wnt11 have prognostic value in AML patients receiving chemotherapy only, but not in AML patients receiving allo-HSCT. The reason may be that allo-HSCT, as a high-intensity treatment, temporarily and fundamentally eliminates some translocations, while chemotherapy cannot achieve such an effect.

Wnt signalling pathway has crucial importance in the pathogenesis of AML. There is heterogeneity in Wnt signalling activation in AML, even in groups with the same genotype.\(^{26}\) Wnt members, including Wnt3, Wnt4, Wnt5B, Wnt6, Wnt7B, Wnt9A, Wnt10A, Wnt14 and Wnt16, are highly expressed in chronic lymphocytic leukaemia B cells.\(^{27,28}\) Nuclear non-phosphorylated beta-catenin (NPBC) is produced by activated Wnt signalling pathway, which is associated with poor survival in AML-M6 and M7 patients.\(^{29}\) However, we found that high Wnt2B expression subgroup has fewer AML-M4 patients, whereas high Wnt10A expression subgroup has more AML-M4 patients in the chemotherapy group, suggesting that different Wnt genes may be selectively expressed in different AML subtypes. We also found that high Wnt11 expression is compatible with some poor prognostic factors (poor risk and IDH1/IDH2 mutations), while high Wnt10A expression is incompatible with typical undesirable clinical features (high WBC count, BM and PB blasts) in the chemotherapy group. AML-associated mutations can activate the downstream Wnt pathway that may contribute to leukaemia pathogenesis.\(^{3}\) For example, NPM1 mutation can activate Wnt signalling by causing the expansion of haematopoietic progenitor cell pool in zebrafish haematopoiesis;\(^{30}\) FLT3-ITD can activate Wnt/\( \beta\)-catenin signalling in primary AML cells.\(^{31}\) However, we did not find that the expression of Wnt2B, Wnt10A and Wnt11 are associated with NPM1
and FLT3-ITD mutations. The small sample size and the influence of other regulatory factors may be responsible for this phenomenon. This also makes it possible for these three Wnt genes to serve as independent prognostic indicators.

Wnt2B is one of the Wnt ligands that stimulate the canonical Wnt pathway. Wnt2B is associated with clinical stage, T stage and cervical lymph node metastasis in patients with nasopharyngeal carcinoma. Wang et al. reported that silencing Wnt2B can decrease the capacity of metastatic dissemination for ovarian cancer cells, and the decreased Wnt2B expression can inhibit cancer cell survival and promote cell apoptosis after chemotherapy treatment. We found that elevated Wnt2B expression is associated with worse survival in AML patients with chemotherapy only. This is similar to previous findings that Wnt2B expression is significantly higher in human pancreatic cancer tissues than in normal pancreatic tissues, and patients with high Wnt2B expression have worse outcomes. Therefore, Wnt2B expression may be used as a new prognostic indicator for AML.

As a member of the canonical Wnt/β-catenin pathway, Wnt10A overexpression is associated with poor survival in patients with idiopathic pulmonary fibrosis. Li et al. observed that overexpression of Wnt10A can promote the occurrence and development of human ovarian cancer by activating the Wnt/β-catenin/TCF/LEF1 signalling pathway. High expression of Wnt10A is associated with high tumour grade and advanced stage in patients with ovarian cancer and may be a predictor of patient survival. Wnt10A expression is related to tumour staging and plays a carcinogenic role in the biological process of colorectal cancer. However, Jiang et al. found that the expression of Wnt10A in colorectal cancer patients is lower than that in the health group. We found that high Wnt10A expression is associated with good outcomes in AML patients with chemotherapy only. This may be due to the heterogeneity of the expression and function of the same gene in different tumours.

Wnt11 activates both canonical and non-canonical Wnt signalling pathways and plays controversial role in different cancers. On the one hand, Wnt11, as a tumour promoter, is involved in the proliferation, migration and invasion of various cancers, such as breast cancer, colon cancer, prostate cancer and leukaemia. On the other hand, Wnt11 is considered to be a tumour-suppressor protein that inhibits tumour cell migration, invasion and adhesion in ovarian cancer, endometrial cancer and liver cancer. We found that high Wnt11 expression indicates poor outcomes in AML patients with chemotherapy only, suggesting that high Wnt11 expression may be involved in leukaemogenesis as a tumour promoter.

We found that the prognostic value for Wnt2B is still significant, but not for Wnt11 in the verification cohort lacking Wnt10A data. The difference in Wnt11 results between the validation cohort and our main analysis may be caused by heterogeneity of the two data sets and incomplete information of the validation cohort (no division of chemotherapy and transplantation groups). Moreover, in the subsequent multivariate analysis, Wnt10A and Wnt11 still have significant independent prognostic value even when competing with typical prognostic factors (age ≥ 60 years, BM blasts ≥ 70% and mutations in DNMT3A, RUNX1, TP53 and MLL) in AML patients with chemotherapy only. Further combined survival analysis also confirmed that AML patients with high Wnt10A and low Wnt11 expression have the longest EFS and OS in the chemotherapy group, suggesting that the combination of Wnt10A and Wnt11 may be a novel prognostic marker for AML and superior to single Wnt gene.

### Table 3

| Variables                          | EFS                      | OS                      |
|-----------------------------------|--------------------------|-------------------------|
|                                   | HR (95%CI)               | P-value                 | HR (95%CI)               | P-value                 |
| Wnt2B (high vs. low)              | 2.100 (1.214, 3.632)     | 0.008                   | NS                       |                             |
| Wnt10A (high vs. low)             | 0.560 (0.323, 0.969)     | 0.038                   | 0.514 (0.296, 0.893)     | 0.018                    |
| Wnt11 (high vs. low)              | 2.550 (1.421, 4.574)     | 0.002                   | 2.750 (1.544, 4.897)     | 0.001                    |
| Age (≥60 vs. <60 years)           | 3.024 (1.584, 5.773)     | 0.001                   | 2.392 (1.253, 4.569)     | 0.008                    |
| WBC (≥15 vs. <15 × 10 /L)         | 1.823 (1.033, 3.217)     | 0.038                   | NS                       |                             |
| BM blasts (≥70 vs. <70%)          | 2.429 (1.373, 4.296)     | 0.002                   | 2.033 (1.159, 3.565)     | 0.013                    |
| PB blasts (≥20 vs. <20%)          | NS                       | NS                      | NS                       |                             |
| FLT3-ITD (positive vs. negative)  | NS                       | NS                      | NS                       |                             |
| NPM1 (mutated vs. wild)           | NS                       | NS                      | NS                       |                             |
| DNMT3A (mutated vs. wild)         | 2.486 (1.372, 4.505)     | 0.003                   | 2.487 (1.384, 4.469)     | 0.002                    |
| IDH1/IDH2 (mutated vs. wild)      | NS                       | NS                      | NS                       |                             |
| RUNX1 (mutated vs. wild)          | 3.505 (1.651, 7.438)     | 0.001                   | 3.292 (1.584, 6.842)     | 0.001                    |
| NRAS/KRAS (mutated vs. wild)      | NS                       | NS                      | NS                       |                             |
| TET2 (mutated vs. wild)           | NS                       | NS                      | 0.536 (0.246, 1.170)     | 0.118                    |
| TP53 (mutated vs. wild)           | 3.136 (1.370, 7.179)     | 0.007                   | 2.497 (1.139, 5.473)     | 0.022                    |
| MLL (mutated vs. wild)            | 2.909 (1.325, 6.387)     | 0.008                   | 2.920 (1.305, 6.535)     | 0.009                    |

Abbreviations: BM, bone marrow; CI, confidence interval; EFS, event-free survival; HR, hazard ratio; OS, overall survival; PB, peripheral blood; WBC, white blood cell.
Pathway enrichment analysis showed that Wnt10A and Wnt11 may participate in the biological process of AML by interacting with genes in 'cell morphogenesis involved in differentiation', 'blood vessel development', 'haemostasis' and 'haematopoietic cell lineage' signalling pathways. The specific joint mechanism of them in AML needs to be further clarified.

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CONFLICT OF INTEREST
The authors confirm that there are no conflicts of interest.
AUTHOR CONTRIBUTIONS
Yifeng Dai and Zhiheng Cheng: collected and analysed the data. Yifeng Dai: drafted the manuscript. Zhiheng Cheng: revised the manuscript. Doerte R. Fricke, Hongyou Zhao, Wenhui Huang, Qingfu Zhong, Pei Zhu, Wenjuan Zhang, Zhihua Wu, Qing Lin, Huoyuan Zhu, Yan Liu and Tingting Qian: participate in the data collection and discussion in the revision. Lin Fu, Longzhen Cui and Tiansheng Zeng: conceived and led the study. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENT
All data sets are available through the Cancer Genome Atlas (TCGA) data portal (https://tcga-data.nci.nih.gov/tcga). We do not involve direct sample collection. All data analyses of this study have been included in the article.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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