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**Prognostic role of ring finger and WD repeat domain 3 and immune cell infiltration in hepatocellular carcinoma**

Miao YD *et al.* RFWD3 in HCC

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**Abstract**

The expression of ring finger and WD repeat domain 3 (RFWD3) is significantly higher in unpaired and paired hepatocellular carcinoma (HCC) tissues than in normal tissues. The expression of RFWD3 has a significant correlation with the infiltration level of 14 immune cells. There is a prominent difference in overall survival, disease-specific survival, and progression-free interval between the high and low RFWD3 expression subgroups. Besides, univariate cyclooxygenase analysis indicated that RFWD3 is an independent prognostic element for HCC. RFWD3 has an ability to accurately predict the prognosis in HCC (area under curve value = 0.863). Furthermore, nomogram can predict the probabilities of 1-, 3- and 5-year osteosarcoma *via* integrating the age, pathologic stage, AFP, and RFWD3 expression in HCC.

**Key Words:** Hepatocellular carcinoma; Ring finger and WD repeat domain 3; Immune cell infiltration; Bioinformatics

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**Core Tip:** We discovered that ring finger and WD repeat domain 3 (RFWD3) expression is remarkable higher in tumor group than in the corresponding normal group, whether in unpaired or paired hepatocellular carcinoma (HCC) tissues. RFWD3 expression has a significant correlation with the infiltration level of 14 immune cells. Besides, univariate cyclooxygenase regression analysis indicated that RFWD3 is an independent prognostic element in HCC. RFWD3 has the ability to accurately predict the prognosis in HCC.

**TO THE EDITOR**

We studied with an interesting paper by Liang *et al*[1]. They assessed ring finger and WD repeat domain 3 (RFWD3) expression levels in hepatocellular carcinoma (HCC) patients. Furthermore, they found RFWD3 affects the prognosis, proliferation, invasion, and metastasis of HCC *via* regulating the Wnt/β-catenin signal pathway. We appreciate the authors' unique view in probing into the prognostic implication of RFWD3 in HCC. We also discovered the expression of RFWD3 was prominently higher in both unpaired and paired HCC tissues than in the corresponding normal tissues (Figure 1A and B). According to the current research, cancer cells, endothelial cells, stromal cells, immune cells, and cancer-associated fibroblasts cells all exist in the tumor microenvironment (TME)[3,4]. TME is also an crucial part in the development, invasion and metastasis of HCC, however, immune escape of HCC has become a hard conundrum in cancer treatment[5]. Due to the limitations of chemotherapy in the liver cancer therapy, many immunotherapy for liver cancer arises at the historic moment. Immunotherapy mainly uses immune cells inside and outside TME to specific target and attack cancer cells, which has an advantage of high specificity and low side-effect[6]. Different types of immune-related cells act diverse roles, containing inhibiting/promoting HCC and varying types that are controversial[7]. Liang *et al*[1] found RFWD3 affects the prognosis of HCC. Therefore, we tested a hypothesis that the expression of RFWD3 maybe associate with the immune cells infiltration in HCC. Detailed information are shown in Table 1.

To confirm the hypothesis, in this research, we explored the correlation between RFWD3 expression and infiltration level of 24 immune cells by spearman's analysis using ssGSEA algorithm[8]. We found that RFWD3 expression has a remarkable correlation with the infiltration level of 14 immune cells (Figure 2A), among them, RFWD3 expression is positively associated with the infiltration level of Th2 cells, T helper cells, T follicular helper (TFH) cells, T central memory (Tcm) cells, activated dendritic cell (DC), NK CD56 bright cells, Eosinophils cells (Figure 2B-H, all *P* < 0.05); and negatively associated with cytotoxic cells, DC, plasmacytoid DC (pDC), Neutrophils cells, T gamma delta cells (Tgd), Treg cells, Th17 cells (Figure 2I-O, all *P* < 0.05). These finding might encourage further detection the effection of RFWD3 in HCC immunotherapy. Detailed information is manifested in Table 2.

We agree with Liang *et al*[1]*,* who found that higher RFWD3 expression show a worse OS in HCC. Our study found that OS, DSS, and PFI are prominently shorter in the high RFWD3 subgroup than in the low RFWD3 subgroup (Figure 3A-C, all *P* < 0.05). Besides, univariate cyclooxygenase analysis demonstrated that RFWD3 is an independent prognostic element for HCC patients (Table 3). Receiver operating characteristic curve showed that RFWD3 has the ability to accurately predict the prognosis in HCC (area under curve = 0.863). Furthermore, we utilized the nomogram to predict the probabilities of 1-, 3- and 5-year osteosarcoma *via* integrating age, pathologic stage, AFP, and RFWD3 expression. Each element was assigned a score in ratio to its contribution to the survival risk (Figure 3E). Conversely, the above fingding indicated that the research performed through Liang *et al*[1] is worthy of attention and that our discover may be provide supplementary information to their study, which can make the study more significant.

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**Footnotes**

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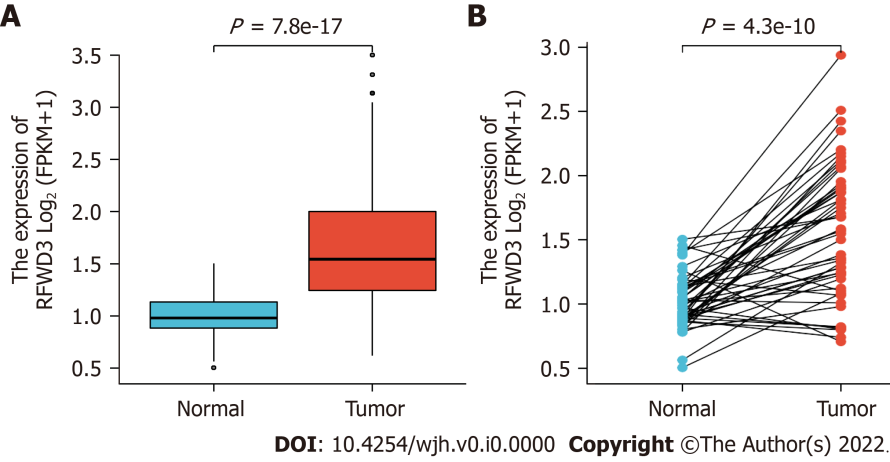
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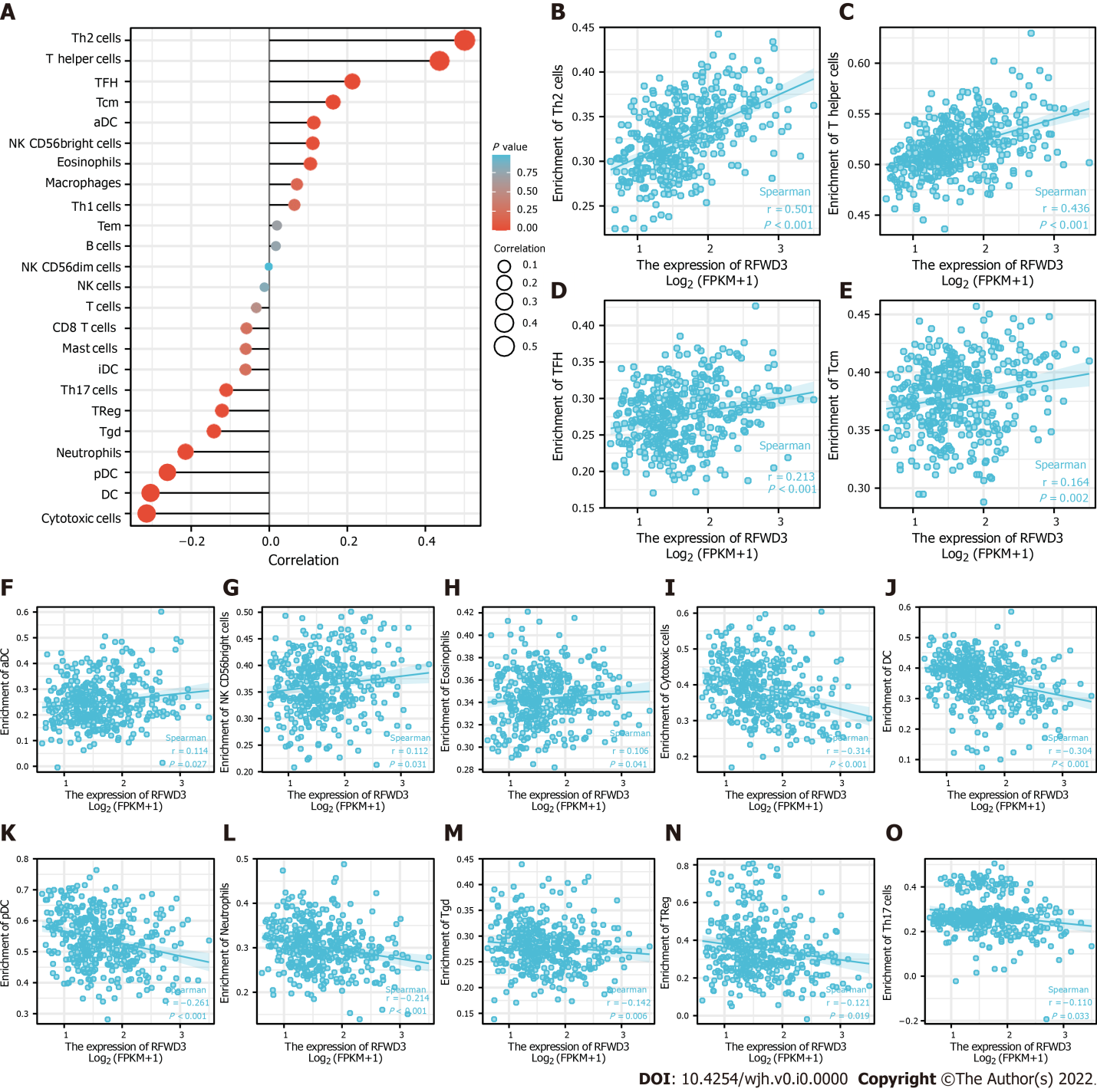
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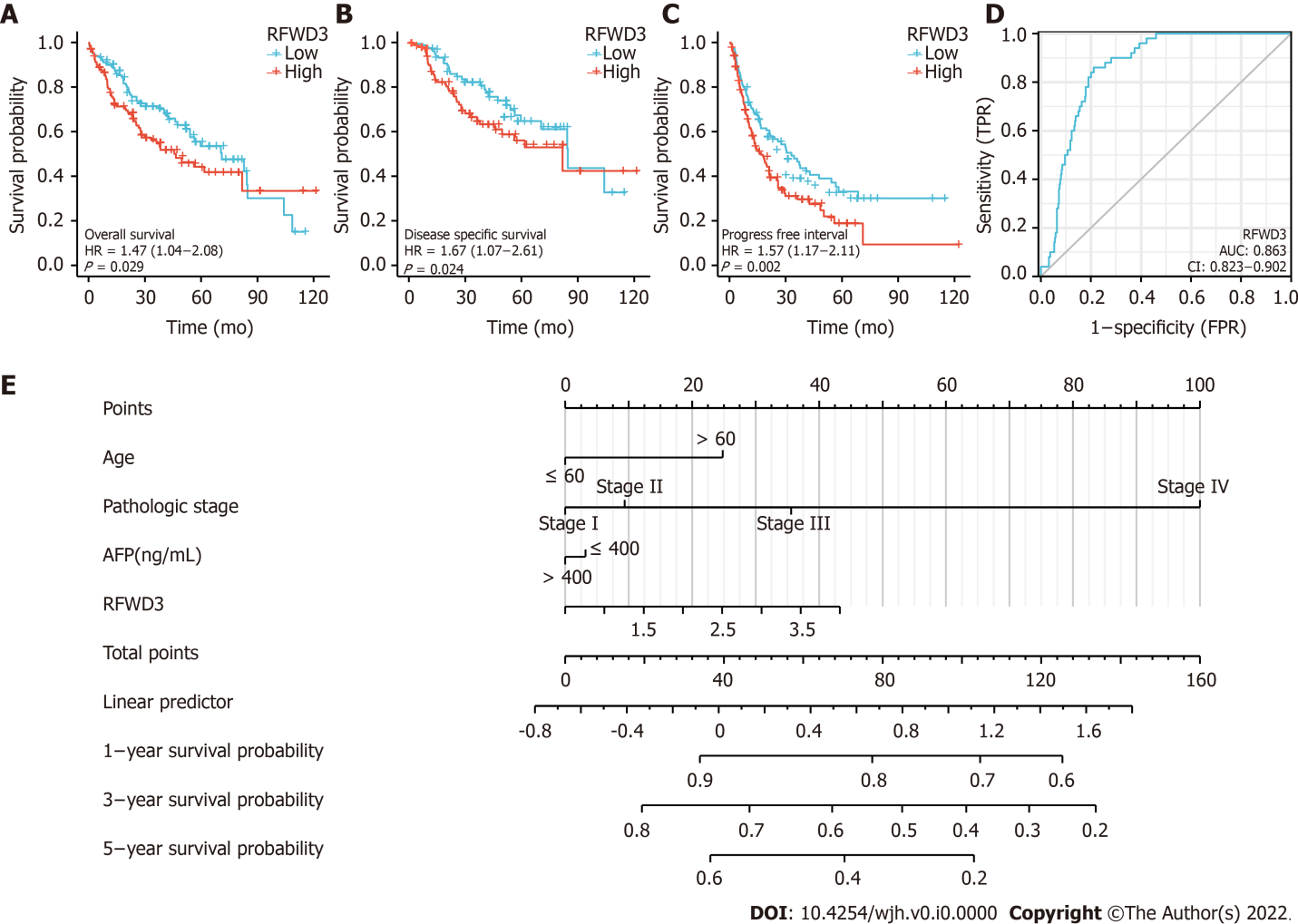
**Figure Legends**



**Figure 1 The differential expression level of** **ring finger and WD repeat domain 3 in hepatocellular carcinoma and normal tissues.** A: Non-paired hepatocellular carcinoma (HCC) and normal samples; B: Paired HCC and normal samples. Data source: mRNA seq data of tetraphenylmethane format for GTEx and Cancer Genome Atlas processed consistently through the Toil process in the UCSC XENA database.



**Figure 2 Correlation analysis of ring finger and WD repeat domain 3 expression and immune cell infiltration in hepatocellular carcinoma.** A: Lollipop plot manifesting the correlation between ring finger and WD repeat domain 3 (RFWD3) expression and the infiltration level of 24 immune cells; B-H: Seven immune cell infiltration levels have significant positive correlation with RFWD3 expression; B: Th2 cells; C: T helper cells; D: T follicular helper cells; E: T central memory cells; F: Activated dendritic cell; G: Natural killer CD56 bright cells; H: Eosinophils cells; I-O: Seven immune cell infiltration levels have significant negative correlation with RFWD3 expression; I: Cytotoxic cells; J: Dendritic cell; K: Plasmacytoid dendritic cell; L: Neutrophils cells; M: T gamma delta; N: Treg cells; O: Th17 cells. aDC: Activated dendritic cell; DC: Dendritic cell; iDC: Immature dendritic cell; NK: Natural killer; pDC: Plasmacytoid dendritic cell; Tcm: T central memory; Tem: T effector memory; Tfh: T follicular helper; Tgd: T gamma delta.



**Figure 3 Survival analysis of ring finger and WD repeat domain 3 in hepatocellular carcinoma and the nomogram.** Ring finger and WD repeat domain 3 (RFWD3) expression is related to A: Overall survival; B: Disease-specific survival; and C: progression-free interval in the Cancer Genome Atlas-hepatocellular carcinoma (HCC); D: Receiver operating characteristic curves of RFWD3 gene predicting prognosis. Nomogram can predict 1-, 3-, and 5-year osteosarcoma of HCC based on clinicopathological features and the expression of RFWD3.

**Table 1 Detailed statistical results of differential expression of ring finger and WD repeat domain 3 in hepatocellular carcinoma**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Group** | **Number** | **Minimum** | **Maximum** | **Median** | **IQR** | **Lower quartile** | **Upper quartile** | **Mean** | **SD** | **SE** |
| RFWD3 | Normal | 50 | 0.504 | 1.504 | 0.98 | 0.251 | 0.883 | 1.133 | 1.013 | 0.208 | 0.029 |
| RFWD3 | Tumor | 374 | 0.62 | 3.5 | 1.544 | 0.755 | 1.245 | 2 | 1.647 | 0.559 | 0.029 |
| RFWD3 | Normal | 50 | 0.504 | 1.504 | 0.98 | 0.251 | 0.883 | 1.133 | 1.013 | 0.208 | 0.029 |
| RFWD3 | Tumor | 50 | 0.707 | 2.939 | 1.577 | 0.716 | 1.204 | 1.92 | 1.578 | 0.521 | 0.074 |

RFWD3: Ring finger and WD repeat domain 3; IQR: Interquartile distance; SD: Standard deviation; SE: Standard error.

**Table 2 Detailed information on the correlation between ring finger and WD repeat domain 3 expression and immune cell infiltration**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Cell** | **Correlation coefficient (Pearson)** | ***P* value**  **(Pearson)** | **Correlation coefficient (Spearman)** | ***P* value**  **(Spearman)** |
| RFWD3 | Th2 cells | 0.499 | < 0.001 | 0.501 | < 0.001 |
| T helper cells | 0.434 | < 0.001 | 0.436 | < 0.001 |
| Cytotoxic cells | -0.304 | < 0.001 | -0.314 | < 0.001 |
| DC | -0.281 | < 0.001 | -0.304 | < 0.001 |
| pDC | -0.261 | < 0.001 | -0.261 | < 0.001 |
| Neutrophils | -0.210 | < 0.001 | -0.214 | < 0.001 |
| TFH | 0.226 | < 0.001 | 0.213 | < 0.001 |
| Tcm | 0.187 | < 0.001 | 0.164 | 0.002 |
| Tgd | -0.105 | 0.043 | -0.142 | 0.006 |
| TReg | -0.155 | 0.003 | -0.121 | 0.019 |
| aDC | 0.141 | 0.006 | 0.114 | 0.027 |
| NK CD56bright cells | 0.128 | 0.013 | 0.112 | 0.031 |
| Th17 cells | -0.170 | < 0.001 | -0.110 | 0.033 |
| Eosinophils | 0.077 | 0.135 | 0.106 | 0.041 |
| Macrophages | 0.096 | 0.063 | 0.071 | 0.171 |
| Th1 cells | 0.090 | 0.081 | 0.064 | 0.214 |
| iDC | -0.034 | 0.507 | -0.061 | 0.241 |
| Mast cells | -0.053 | 0.309 | -0.060 | 0.247 |
| CD8 T cells | -0.047 | 0.368 | -0.058 | 0.260 |
| T cells | -0.013 | 0.796 | -0.033 | 0.522 |
| Tem | 0.085 | 0.100 | 0.020 | 0.704 |
| B cells | 0.033 | 0.525 | 0.017 | 0.744 |
| NK cells | 0.035 | 0.494 | -0.012 | 0.810 |
| NK CD56dim cells | 0.020 | 0.699 | -0.001 | 0.979 |

RFWD3: Ring finger and WD repeat domain 3; a DC: Activated dendritic cell; DC: Dendritic cell; i DC: Immature dendritic cell; NK: Natural killer; p DC: Plasmacytoid dendritic cell; Tcm: T central memory; Tem: T effector memory; Tfh: T follicular helper; Tgd: T gamma delta.

**Table 3 Univariate cyclooxygenase regression analysis in hepatocellular carcinoma**

| **Characteristics** | **Total (*n*)** | **Univariate analysis** | |
| --- | --- | --- | --- |
| **Hazard ratio (95%CI)** | ***P* value** |
| Pathologic stage | 349 |  |  |
| Stage I | 173 |  |  |
| Stage II | 86 | 1.417 (0.868-2.312) | 0.164 |
| Stage III | 85 | 2.734 (1.792-4.172) | < 0.001 |
| Stage IV | 5 | 5.597 (1.726-18.148) | 0.004 |
| Child-Pugh grade | 240 |  |  |
| A | 218 |  |  |
| B | 21 | 1.595 (0.757-3.361) | 0.219 |
| C | 1 | 2.138 (0.294-15.544) | 0.453 |
| Fibrosis ishak score | 214 |  |  |
| 0 | 75 |  |  |
| 1/2 | 31 | 0.935 (0.437-2.002) | 0.864 |
| 3/4 | 28 | 0.698 (0.288-1.695) | 0.428 |
| 5/6 | 80 | 0.737 (0.410-1.325) | 0.308 |
| Histologic grade | 368 |  |  |
| G1 | 55 |  |  |
| G2 | 178 | 1.162 (0.686-1.969) | 0.576 |
| G3 | 123 | 1.185 (0.683-2.057) | 0.545 |
| G4 | 12 | 1.681 (0.621-4.549) | 0.307 |
| RFWD3 | 373 | 1.557 (1.148-2.110) | 0.004 |