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HHLA2 overexpression promoted colorectal cancer growth by enhancing glycosylation and weakening the binding of HHLA2 to KIR3DL3

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Abstract:

Colorectal cancer (CRC) ranks as the third most common cause of cancer-related fatalities globally, representing a significant public health challenge. The highest rates are reported in North America, Oceania, and Europe, particularly Eastern Europe, which may be related to "Westernized" lifestyles such as obesity and physical inactivity. In contrast, the incidence is relatively low in Asia, Africa, and South America. Colorectal cancer mortality is declining in many developed and emerging economies, linked to improved screening and treatment. However, in certain resource-limited countries like Mexico, Brazil, Romania, and Russia, mortality rates continue to rise. This suggests that despite advances in certain countries, colorectal cancer remains a serious global public health challenge and that a massive scale-up of targeted screening programs is needed to reduce its burden. HHLA2 is abundantly expressed in colorectal cancer tissues and inversely correlated with the presence of anti-tumor immune cells, indicating that it could have a dual function in both enhancing immune checkpoints and inhibiting immune responses. In addition, a change in the N-glycosylation pattern has been observed in colorectal cancer. It is closely related to the abnormal expression of glycosyltransferases. Further research on the mechanism of HHLA2 and N-glycosylation on the development and progression of colorectal cancer will help to better understand the role of these factors in colorectal cancer and provide new ideas for treatment.

Keywords: HHLA2, Glycosylation, Colorectal cancer, Immune checkpoints, Natural killer cells

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1. Introduction

Colorectal cancer is the 3rd most common factor of cancer-related deaths around the world and poses a significant public health challenge. [1]

Colorectal cancer is among the malignancies associated with high rates of morbidity and mortality globally. [2] In many developed and emerging economies, colorectal cancer mortality is on a downward trend, which is associated with improved screening and treatment. However, in some countries with limited resources, such as Mexico, Brazil, Romania and Russia, death rates are still rising. These data show that despite progress in some countries, colorectal cancer remains a serious global public health challenge, and scaling up targeted screening programmes can reduce its burden.[3]

After investigation, HHLA2 plays a crucial role in colorectal cancer research. Its expression levels were noteworthy elevated in tumor tissues of colorectal cancer compared to normal tissues, and immunohistochemical analysis revealed that 97% of tumor samples exhibited HHLA2 expression. [2] HHLA2 expression is negatively correlated with the expression of both anti-tumor cytokines and pro-tumor growth factors, indicating that HHLA2 may have a dual regulatory function in colorectal cancer, both stimulating immune checkpoints and suppressing immune response.[2]

HHLA2 was found to be abundantly expressed in colorectal cancer tissues and linked to larger tumor size and worse prognosis. Its expression showed a negative correlation with the count of NK cells and macrophages, but no significant correlation with activated CD4+ T cells, CD8+ T cells, B cells, or dendritic cells. [1] HHLA2 restrains the anti-tumor immune function of NK cells in the way of binding to the inhibitory receptor KIR3DL3, thus facilitating the advancement of colorectal cancer. [1]

HHLA2 shows a complex mechanism of action in colorectal cancer, and its potential value in promoting tumor development and inhibiting tumor progression warrants further study in order to afford new ideas for the therapy of colorectal cancer.

Simultaneously, N-glycosylation undergoes notable alterations during the onset and progression of colorectal cancer. In colorectal cancer, the N-glucoside structure of high mannose-type, The hybrid type and oligomannose type were significantly elevated, whereas the complex type showed a decrease. [4] There was a significant increase in α2, 6-sialylation, while α2, 3-sialylation experienced a decline. In cases of advanced colorectal cancer, α2, 3-sialylation is increased compared to early stage, while double-branched GlcNAc and Lewis-type glycosylation are decreased.[4] These changes of N-glycosylation are closely related to the abnormal expression of glycosyltransferase. The expression levels of associated glycosyltransferases in colorectal cancer tissues were either upregulated or downregulated, which was correlated with abnormal changes of n-glycosylation. In addition, the change of N-glycosylation is also related to the status of epidermal growth factor receptor (EGFR). In colorectal cancer tissue that is positive for EGFR, double-branched GlcNAc was increased, while α2, 3-sialylation was decreased.[4]

Hypothesis:

Overexpression of HHLA2 in mice leads to increased HCT116 xenograft tumor size, increases the n-glycosylation of HHLA2 and reduces its combining to the inhibitory receptor KIR3DL3, thereby reducing immune of the anti-tumor function of Natural Killer cells.

2. Material and Methods

Measure glycosylation of HHLA2 by western blot and measure binding of HHLA2 to KIR3DL3 by SPR, and NK killing by a NK/HCT116 coculture assay and xenograft tumor size by weight. Positive control for xenograft and coculture experiment is Taxol, negative control is empty expression vector.

The tumor size of HCT116 xenograft in HHLA2-overexpressing mice and wild-type mice (non-artificially mutated, carrying wild-type genome) will be compared.

2.1 . Method 1 Western blot detected the glycosylation level

Cells were cleaved using RIPA buffer and supernatants were collected by centrifugation at 12,000g for 20 min. Protein concentrations were then determined using the BCA protein assay kit and subjected to SDS-PAGE electrophoresis and transfer onto PVDF membranes. Following a 1-hour blocking period with 5% nonfat milk powder at room temperature, the cells were incubated overnight at 4 °C with antibodies targeting HHLA2/B7-H7, DYKDDDDK Tag, HA, STT3A, STT3B, and GAPDH. This was followed by incubation with horseradish peroxidase-labeled secondary antibodies at room temperature and detection of protein bands using ECL reagents.

To detect HHLA2 glycosylation, cells were lysed and boiled in glycoprotein denaturation buffer for 10 min, followed by addition of neuraminidase, O-Glycosidase, Endo H, and PNGase F, respectively, and then incubated overnight at 37 °C for Western blot analysis. Cells were treated at different time points (6h, 12h, and 24h) to observe the changing trend of HHLA2 glycosylation level with time. In this way, we can understand the dynamic process of HHLA2 glycosylation more comprehensively.

The glycosylation status of HHLA2 was examined and we

can use this way to evaluate the effect of N-glycosylation on HHLA2 stability and cell membrane localization. Negative control (empty expression cells) and positive control (Taxol) were set up in the experiment to ensure the reliability of the experimental results.

2.2 . Method 2 SPR detected the binding

HHLA2 protein was covalently coupled to the CM5 chip. KIR3DL3-His protein was incubated in HBS-EP+ buffer at different concentrations (e.g., 5, 10, 20 μg/mL) to flow through the HHLA2 chip, and the binding kinetics of KIR3DL3-His protein to HHLA2 was detected. The binding of HHLA2 and KIR3DL3 was detected at different time points (such as 15 min, 30 min, 1 h, 2 h, and 4 h), and its changing trend with time was observed. This can help to better understand the binding kinetic process of the two. Blank microarrays were set as negative controls to rule out nonspecific binding. Using the HHLA2-8NQ mutant microarray, the ability to bind KIR3DL3 was tested to verify the influence of N-glycosylation on combining. KIR3DL3 antibody was added to KIR3DL3-His protein to test whether the binding of HHLA2 to KIR3DL3 could be blocked and the specificity of binding was confirmed. Other proteins, such as TMIGD2-His, were used as negative controls to test their binding ability to HHLA2.

Using the methods described above, kinetic parameters of HHLA2 binding to KIR3DL3, such as dissociation constant (KD), binding rate constant (ka) and dissociation rate constant (kd), could be quantified, and we can evaluate the influence of N-glycosylation on combining.

2.3 . Method 3 Co-culture experiment

CT116 colon cancer cells and NK-92MI cells were obtained, and cell growth was maintained according to the culture conditions recommended by ATCC. Pre-treated NK-92MI cells were co-cultured with HCT116 cells for 6 hours at various effector to target cell ratios. CFSE was used to label HCT116 cells for subsequent detection of their apoptotic levels. The apoptosis level of HCT116 cells after co-culture was detected by flow cytometry using Annexin V-PE apoptosis detection kit. HCT116 cells that didn't undergo NK cell treatment were designated as the negative control. Additional control groups, including NK cells and HCT116 cells, could be established to confirm the specificity of the results. The levels of Th1/Th2/Th17 cytokines in the co-culture supernatant were detected. The effect of N-glycosylation on HHLA2 function was evaluated, such as using HHLA2-8NQ mutant cells.

More different ratios of effector cells to target cells, such as 1:1, 5:1, 10:1, 20:1, etc., were tried to more comprehensively assess the influence of HHLA2 on Natural Killer cell killing activity. The apoptosis level of HCT116 cells was detected at more time points (such as 2 h, 12 h, and 24 h) to observe the time-dependent effect of HHLA2 on NK cell killing activity. NK-92MI cells were pretreated at different concentrations (such as 20 μM, 40 μM, 80 μM) with PI3K activator 740 Y-P to observe the regulatory effect of HHLA2 on PI3K-Akt signaling pathway.

The killing ability of NK cells against HCT116 cells was comprehensively evaluated, and the role of N-glycosylation of HHLA2 in this process was investigated. The results will provide an important basis for further study of the mechanism of HHLA2 in tumor immune escape.

2.4 . Method 4 transplantation tumor

The RKO-HHLA2-WT, RKO-HHLA2-8NQ, and RKO-Vector cell lines were subcutaneously inoculated with 2×10^6 cells into 6-week-old NSG male nude mice each mouse. Mice were sacrificed and tumor tissue was removed, and tumor weight was measured using an electronic balance.

On days 10, 12, 14, and 16 of inoculation, 20 mg/kg of NC-NPs or NGI-1-NPs were administered intravenously. Different concentrations of N-glycosylation inhibitor NGI-1(such as 10 mg/kg, 20 mg/kg, 40 mg/kg) were used to pretreat the RKO-HHLA2-WT xenograft tumor mice to observe the regulatory influence of N-glycosylation on HHLA2 function.

On days 9, 11, 13 and 15 of inoculation, 1×10^7 NK-92MI cells were injected intravenously into the NC-NPs + NK-92MI and NGI-1-NPs + NK-92MI groups, respectively. Tumor tissue sections were collected and immunohistochemical staining was performed to detect the expression levels of IL-2, TNF-α and IFN-γ. We will use GraphPad Prism 8.0.1 software for statistical analysis, including t test, one-way or two-way analysis of variance, Tukey's multiple comparison test, and Kaplan-Meier survival analysis.

The influence of tumor growing of N-glycosylation of HHLA2 on and NK cell killing was comprehensively evaluated.

2.5 . Statistical Analysis

Data analysis was performed using GraphPad Prism 8.0.1 software. Group differences were compared using t tests or one-way/two-way ANOVA, followed by Tukey's multiple comparison test. For survival analysis, the Kaplan-Meier method with Log-rank test was employed.

Specifically, tumor weights from mice with RKO-HHLA2-8NQ, RKO-Vector, and RKO-HHLA2-WT cells were analyzed using one-way ANOVA and Tukey's test. The difference in HCT116 xenografts between HH-

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LA2-overexpressing and wild-type mice was assessed via t test. Correlation analysis was conducted to evaluate the relationship between HHLA2+ expression and tumor size, as well as patient prognosis, and to examine the association between HHLA2 expression and immune cell quantities (NK cells and macrophages). Kaplan-Meier survival curves were generated to compare survival across groups. Overall, HHLA2 expression's impact on tumor growth and patient prognosis was comprehensively evaluated.

3. Results

Table 1: Possible Combination Results of HHLA2 expression

Combination Result # (CR#)				
	HHLA2 expression increases tumor size of HCT116 xenografts	HHLA2 overexpression increases glycosylation by western blot mass shift	HHLA2 overexpression reduces HHLA2/KIR3DL3 interaction by SPR	Support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	+	Partial
4	-	+	+	Partial
5	+	-	-	Partial
6	-	-	+	Partial
7	-	+	-	Partial
8	-	-	-	Fully Contradicts

Table legend: "+" indicates a result of the index is higher than the positive control (Taxol). "-" indicates a result of the index is lower than the positive control(Taxol).

CR 1: If I obtain this result I would see HHLA2 is abundantly expressed in colorectal cancer tissues and positively correlated with tumor size. However, overexpression of HHLA2 didn't influence the proliferation ability of colorectal cancer cells. By comparison, the experimental group is more glycosylated than the experimental positive control (Taxol). The degree of HHLA2/KIR3DL3 interaction was higher in the experimental group than in the positive control.

CR 2 If I obtain this result I would see HHLA2 is abundantly expressed in colorectal cancer tissues and positively correlated with tumor size. However, overexpression of HHLA2 didn't influence the proliferation ability of colorectal cancer cells. By comparison, the experimental group is more glycosylated than the experimental positive control (Taxol). The degree of HHLA2/KIR3DL3 interaction was much less in the experimental group than in the positive control.

CR 3: If I obtain this result I would see HHLA2 is abundantly expressed in colorectal cancer tissues and positively correlated with tumor size. However, overexpression of HHLA2 didn't influence the proliferation ability of colorectal cancer cells. By comparison, the experimental group is less glycosylated than the experimental positive control (Taxol). The degree of HHLA2/KIR3DL3 interaction was higher in the experimental group than in the positive control.

CR 4: If I obtain this result I would see Overexpression of HHLA2 didn't affect the proliferative capacity of colorectal cancer cells, even in vivo. After comparison, the experimental group is more glycosylated than the experimental positive control (Taxol). The degree of HHLA2/KIR3DL3 interaction was higher in the experimental group than in the positive control.

CR 5: If I obtain this result I would see HHLA2 is abundantly expressed in tissues of colorectal cancer and positively correlated with tumor size. However, overexpression of HHLA2 didn't influence the proliferation capacity of colorectal cancer cells. By comparison, the experimental group is less glycosylated than the experimental positive control (Taxol). The degree of HHLA2/KIR3DL3 interaction was lower in the experimental group than in the positive control.

CR 6: If I obtain this result I would see Overexpression of HHLA2 didn't affect the proliferative capacity of colorectal cancer cells, even in vivo. By comparison, the experimental group is less glycosylated than the experimental positive control (Taxol). The degree of HHLA2/KIR3DL3 interaction was higher in the experimental group than in

the positive control.

CR 7: If I obtain this result I would see Overexpression of HHLA2 didn't affect the proliferative capacity of colorectal cancer cells, even in vivo. By comparison, the experimental group is more glycosylated than the experimental positive control (Taxol). The degree of HHLA2/KIR3DL3 interaction was lower in the experimental group than in the positive control.

CR 8: If I obtain this result I would see Overexpression of HHLA2 didn't affect the proliferative capacity of colorectal cancer cells, even in vivo. By comparison, the experimental group is less glycosylated than the experimental positive control (Taxol). The degree of HHLA2/KIR3DL3 interaction was lower in the experimental group than in the positive control.

4. Discussion

CR1: HHLA2 is significantly upregulated in colorectal cancer, correlating positively with tumor size, indicating its involvement in tumor progression through mechanisms beyond direct cell proliferation. Although HHLA2 overexpression does not enhance cancer cell proliferation, it presents two molecular weight bands (70 kDa and 46.8 kDa), suggesting post-translational modifications, primarily N-glycosylation. Mutating aspartic acid residues to glutamine substantially reduces HHLA2's molecular weight, confirming that N-glycosylation is its primary modification.

Decreased N-glycosylation from HHLA2 overexpression may impair binding to the KIR3DL3 receptor by reducing stability and membrane localization. Consequently, unmodified HHLA2 cannot effectively bind KIR3DL3, leading to inhibited NK cell function, including reduced cytotoxicity. Furthermore, HHLA2 overexpression suppresses the PI3K-AKT signaling pathway in NK cells, further compromising their anti-tumor activity. The immunosuppressive effects of HHLA2 are significantly diminished with KIR3DL3 expression inhibition, underscoring its role in immune escape.

These findings provide crucial insights for investigating HHLA2's mechanisms in tumor immune evasion. Future studies should clarify the specific effects of HHLA2 overexpression on NK cell function, including changes in cytotoxicity and cytokine secretion, as well as its influence on PI3K-AKT signaling.

CR2: HHLA2 overexpression may enhance tumor growth by inhibiting NK cell function. This overexpression decreases HHLA2's N-glycosylation level, reducing its molecular weight and stability, which affects binding to the inhibitory receptor KIR3DL3.

The immunosuppressive effect of HHLA2 on NK cells is significantly diminished with KIR3DL3 expression inhibition, confirming its critical role in tumor immune escape through KIR3DL3 binding. Future studies should verify the specific effects of HHLA2 overexpression on NK cell functions such as cytotoxicity and cytokine output, as well as its mechanism of inhibiting the PI3K-AKT pathway. The influence of HHLA2's N-glycosylation on stability, localization, and KIR3DL3 binding also warrants further investigation, in addition to exploring its potential association with tumor cell migration and invasion through in vitro assays.

CR3: HHLA2 overexpression is linked to increased tumor weight in HCT116 cell xenografts, suggesting its promotion of tumor growth through mechanisms beyond direct proliferation. This overexpression reduces glycosylation levels, alters molecular weight, and impairs binding to KIR3DL3, which may inhibit NK cell function.

Loss of N-glycosylation diminishes HHLA2 stability, making it more prone to ubiquitination and degradation, while hindering its membrane localization. As a result, unglycosylated HHLA2 cannot effectively bind to KIR3DL3. Additionally, HHLA2 overexpression inhibits the PI3K-AKT signaling pathway in NK cells, further undermining their anti-tumor immune responses.

The immunosuppressive effect of HHLA2 on NK cells is significantly reduced when KIR3DL3 expression is inhibited, emphasizing the importance of KIR3DL3 binding in HHLA2-mediated immune evasion. Future studies should investigate specific effects of HHLA2 overexpression on NK cell functions, particularly cytotoxicity and cytokine secretion, as well as elucidate the mechanisms of PI3K-AKT signaling inhibition.

CR4: HHLA2 overexpression leads to reduced tumor weight in HCT116 cell xenografts, suggesting it promotes tumor growth through mechanisms other than direct proliferation. This overexpression increases glycosylation levels, affecting molecular weight and KIR3DL3 receptor binding, which may impact NK cell function. HHLA2 exhibits two molecular weight forms, indicating post-translational modifications. Mutation of these residues to glutamine significantly reduces molecular weight, confirming N-glycosylation as the main modification.

Reduced N-glycosylation decreases HHLA2 stability and membrane localization, impairing KIR3DL3 binding. Consequently, unglycosylated HHLA2 cannot inhibit NK cell function effectively, resulting in decreased cytotoxicity. Moreover, HHLA2 overexpression inhibits the PI3K-AKT signaling pathway in NK cells, further compromising their anti-tumor immune function. The immunosuppressive effect of HHLA2 on NK cells is significantly weakened when KIR3DL3 expression is inhibited, highlighting the crucial role of KIR3DL3 binding in HH-

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LA2-mediated immune

CR5: HHLA2 overexpression in HCT116 xenografts correlates with increased tumor weight, indicating it promotes tumor growth through mechanisms beyond direct proliferation. This overexpression reduces HHLA2 glycosylation, affecting its molecular weight and binding to KIR3DL3 receptors, potentially impairing NK cell function.

Loss of N-glycosylation destabilizes HHLA2, increasing its susceptibility to ubiquitination and degradation while hindering membrane localization. Consequently, unglycosylated HHLA2 cannot effectively bind KIR3DL3, diminishing its capacity to inhibit NK cell functions, including cytotoxicity. Additionally, HHLA2 overexpression inhibits the PI3K-AKT signaling pathway in NK cells, further compromising their anti-tumor responses. The immunosuppressive effect of HHLA2 on NK cells is significantly reduced with KIR3DL3 expression inhibition, highlighting the importance of KIR3DL3 binding in immune evasion.

Future investigations should focus on the specific impacts of HHLA2 overexpression on NK cell functions, examining changes in cytotoxicity and cytokine production, as well as mechanisms of PI3K-AKT signaling inhibition. Additionally, the influence of N-glycosylation on HHLA2's stability, localization, and KIR3DL3 binding warrants further exploration. The potential association of HHLA2 with tumor cell migration and invasion should also be examined through in vitro assays such as scratch and Transwell assays.

CR6: In HCT116 cell xenografts, HHLA2 overexpression is associated with reduced tumor weight, suggesting promotion of tumor growth through non-proliferative mechanisms. This overexpression decreases glycosylation levels, affecting HHLA2's molecular weight and its binding to KIR3DL3 receptors, potentially impairing NK cell function.

The loss of N-glycosylation destabilizes HHLA2, increasing its susceptibility to degradation and hindering its membrane transport. Therefore, unglycosylated HHLA2 is unable to bind KIR3DL3 effectively, compromising NK cell functions, including cytotoxicity. Furthermore, HHLA2 overexpression inhibits the PI3K-AKT signaling pathway in NK cells, significantly weakening their anti-tumor responses. The immunosuppressive impact of HHLA2 diminishes when KIR3DL3 expression is inhibited, underscoring its dependency on KIR3DL3 binding.

Future studies should verify the impacts of HHLA2 overexpression on NK cell function, particularly regarding cytotoxicity and cytokine production, and explore the mechanisms underlying PI3K-AKT signaling inhibition. Additionally, further investigation into N-glycosylation's effects on HHLA2's stability, localization, and KIR3DL3 binding is warranted to clarify its role in tumor immune escape.

CR7: HHLA2 overexpression in HCT116 xenografts is linked to reduced tumor weight, suggesting it promotes tumor growth through mechanisms beyond proliferation. This overexpression decreases glycosylation levels, affecting HHLA2's molecular weight and inhibiting KIR3DL3 receptor binding, which may impair NK cell function.

Loss of N-glycosylation reduces HHLA2 stability, increasing susceptibility to ubiquitination and degradation, while hindering its transport to the membrane. Consequently, unglycosylated HHLA2 cannot effectively bind KIR3DL3, undermining its ability to inhibit NK cell functions, including cytotoxicity.

Additionally, HHLA2 overexpression inhibits the PI3K-AKT signaling pathway in NK cells, further compromising their anti-tumor immune responses. The immunosuppressive effects of HHLA2 on NK cells are significantly reduced with KIR3DL3 expression inhibition, emphasizing the importance of this binding interaction.

Future research should focus on the effects of HHLA2 overexpression on NK cell functions, particularly cytotoxicity and cytokine secretion, and the mechanisms through which it inhibits the PI3K-AKT pathway. Clarifying how N-glycosylation influences HHLA2's stability, localization, and KIR3DL3 binding is also essential. Additionally, the relationship between HHLA2 and tumor cell migration and invasion should be explored through in vitro scratch and Transwell assays.

CR8: HHLA2 overexpression in HCT116 xenografts correlates with reduced tumor weight, indicating a promotion of tumor growth through mechanisms other than direct proliferation. This overexpression decreases HHLA2 glycosylation, affecting its molecular weight and binding to KIR3DL3 receptors, thereby potentially impairing NK cell function.

Loss of N-glycosylation diminishes HHLA2 stability, increasing its susceptibility to degradation and hindering transport to the cell membrane. Thus, unglycosylated HHLA2 fails to effectively bind KIR3DL3, limiting its capacity to inhibit NK cell functions, including cytotoxicity and. Moreover, HHLA2 overexpression inhibits the PI3K-AKT signaling pathway in NK cells, further compromising their anti-tumor responses. The immunosuppressive effects of HHLA2 are significantly reduced when KIR3DL3 expression is inhibited, underscoring the critical role of KIR3DL3 binding.

Future studies should investigate how HHLA2 overexpression specifically impacts NK cell functions regarding cytotoxicity and cytokine secretion, alongside the mechanisms inhibiting the PI3K-AKT signaling pathway. Fur-

ther exploration of N-glycosylation's impact on HHLA2's stability, cellular localization, and KIR3DL3 binding is warranted. Additionally, examining HHLA2's relationship with tumor cell migration and invasion through in vitro assays should be prioritized. Co-culture experiments may validate the effects of HHLA2 overexpression on NK cytotoxicity and cytokine secretion.

5. Conclusions

Colorectal cancer is an important global public health problem with high morbidity and mortality. Despite reductions in mortality due to improved screening and treatment in some developed and emerging economies, mortality rates are still increasing in resource-limited countries such as Mexico, Brazil, Romania, and Russia. This suggests that colorectal cancer remains a major challenge and that screening programs need to be scaled up to reduce its burden.

HHLA2 protein plays an important role in the development of colorectal cancer. Its high expression in tumor tissues is associated with the reduction of anti-tumor immune cells, suggesting that it may play a dual role in immune checkpoint promotion and immune response inhibition. In addition, the changes of N-glycosylation pattern in colorectal cancer are closely related to the expression of abnormal glycosyltransferases.

There is significant heterogeneity in the prognostic significance of HHLA2 in different tumor types. Hhla2 has a protective effect in some tumors, but has no significant prognostic significance or unfavorable prognosis in other tumors.[5] This may be related to the specific functions of HHLA2 in different tumor microenvironments.

As a newly discovered immune checkpoint molecule, the immune function of HHLA2 remains heterogeneous and controversial.[5] Some studies have suggested that HHLA2 has a co-stimulatory effect, promoting T cell proliferation and secretion of related cytokines, while others have suggested that HHLA2 has a co-inhibitory effect, inhibiting the anti-tumor function of T cells.[5]

HHLA2 is widely overexpressed in a variety of human tumors, including breast cancer, lung cancer, and thyroid cancer.[6] HHLA2 may be important in tumor development and provide a survival advantage to tumors by suppressing host anti-tumor immune functions.[6] HHLA2 may promote tumor escape from immune surveillance by inhibiting T cell function.[6] This is consistent with my hypothesis, suggesting that this hypothesis deserves further exploration.

HHLA2 may play different immunomodulatory functions in different tumor types, which may have either co-stimulatory or co-inhibitory effects.[7] This may be related to the glycosylation status of HHLA2 or the interaction with other receptors.[7] The expression of HHLA2 may be an "adaptive immune resistance" response of tumor cells to tumor-infiltrating T cells.[7] This implies that HHLA2 expression may be a mechanism by which tumor cells evade immune attack.

HHLA2 was discovered as B7 family protein. These protein can inhibit the function of human CD4 and CD8 T cells. HHLA2 may promote tumor growth by restraining the immune function of T cells.[8] The paper also states that HHLA2 is not expressed in mice,[8] making it the first member of the B7 family to be expressed only in humans. This means that mouse models may not fully reflect the role of HHLA2 in humans.

In the mouse HCT116 xenograft tumor model, the overexpression of HHLA2 may lead to the increase of its glycosylation level and weaken the combinding between HHLA2 and inhibitory receptor KIR3DL3, [9] thereby reducing the anti-tumor immune function of NK cells and promoting tumor growth. [9] By regulating the glycosylation level of immune checkpoint molecules, it may become a new strategy for tumor immunotherapy. Further study on the effect of HHLA2 glycosylation on its binding to inhibitory receptors and the specific mechanism of tumor immune escape will help to understand the new mechanism of tumor immune escape and provide new targets for clinical treatment.[9]

Further study on the mechanism of HHLA2 and N-glycosylation in the development of colorectal cancer can help to understand their roles in pathology and provide new ideas for treatment. For example, the effect of HHLA2 on tumor cell migration and invasion was detected in vitro, and its inhibition of natural killer cell function. Meanwhile, To investigate the effects of the stability in N-glycosylation, KIR3DL3 receptor binding of HHLA2 and cellular localization.

In conclusion, HHLA2 and N-glycosylation play key roles in colorectal cancer, and in-depth study of these mechanisms will help to better understand the disease and provide new clinical therapeutic targets.

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