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Selective autophagy of NLRC5 promotes immune evasion of endometrial cancer

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ABSTRACT

Endometrial cancer (EC), the most common gynecological cancer, is usually resistant to chemotherapy when the EC patients are advanced or recurrent. Immunotherapy is a promising approach to treat advanced or recurrent EC patients. The innate immune molecule NLRC5 (NLR family CARD domain containing 5) is a major histocompatibility complex class I (MHC-I) transactivator, which is intimately associated with tumor antigen presentation. The absence of NLRC5 expression in cancer results in immune evasion and resistance to immunotherapy. Previously, we found that NLRC5 was downregulated in EC patients, suggesting that NLRC5 is a target for immune evasion in EC. In our recent study, we indicated that autophagy inhibits NLRC5 and NLRC5-mediated MHC-I gene expression *in vitro*. Of special note is that autophagy protein MAP1LC3/LC3 interacts with NLRC5 to inhibit the NLRC5-mediated MHC-I antigen presentation pathway *in vitro* and *in vivo*, which presents a novel mechanism underlying NLRC5-mediated immune evasion by autophagy in EC. Our results reveal a previously unknown mechanism of autophagy protein LC3 in the regulation of NLRC5-mediated MHC-I antigen presentation in EC, and highlight a potential immunotherapy approach in EC patients by inhibiting LC3 and promoting NLRC5.

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NLRC5 as an MHC-I transactivator is well known as a new target for immune evasion in cancer. Furthermore, recent evidence has revealed that autophagy plays a negative role in MHC-I antigen presentation in cancer. Therefore, we explored the related mechanism underlying NLRC5-mediated immune surveillance in EC [1]. We found autophagy is upregulated and MHC-I-related genes are downregulated in EC patient specimens. In EC cells, the autophagy activator rapamycin suppresses the expression of MHC-I-related genes, whereas the autophagy suppressor chloroquine induces the opposite effect, indicating autophagy also plays a negative role in MHC-I-related gene expression in EC. However, the underlying mechanism of the negative role of autophagy in this context was unclear. Because NLRC5 is an MHC-I transactivator, and we found NLRC5 is also downregulated in EC patient specimens, we hypothesized that NLRC5 is involved in this negative role of autophagy; however, it was not known which autophagy protein plays a role in NRLC5 regulation.

We found that autophagy protein LC3 and NLRC5 are colocalized in the cytoplasm of EC cells, and the colocalization is significantly decreased when NLRC5 is knocked down. Furthermore, we revealed an interaction between LC3 and NLRC5 *in vitro*. Additionally, we demonstrated that there is a negative correlation between NLRC5 and LC3 in EC patient specimens. In EC cells, LC3 negatively regulates the expression of NLRC5 and NLRC5-mediated MHC-I-related genes. Taken together, these data suggest that the negative role of autophagy in MHC-I antigen presentation may be involved in restricting NLRC5-mediated MHC-I antigen presentation by LC3 in EC cells.

Indeed, in a system with LC3-overexpressing EC cells co-cultured with $CD8^+$ T cells, we found $CD8^+$ T cell activation is inhibited and the lysis of EC cells by $CD8^+$ T cells is also restricted. When LC3 is inhibited, the appearance of EC cells and $CD8^+$ T cells is reversed. Furthermore, overexpression of NLRC5 elevates the effect on HEC-1A cells by $CD8^+$ T cells, and impairs the effect of LC3 in inhibiting the cytotoxicity of $CD8^+$ T cells in the EC cells co-cultured with $CD8^+$ T cells system. Using xenograft experiments in BALB/C mice, we further revealed that overexpression of LC3 promotes tumor volume and weight, whereas upregulation of NLRC5 expression inhibits tumor volume and weight, and restricts the tumor growth by LC3 *in vivo*. In addition, overexpression of LC3 inhibits the expression of NLRC5 and MHC-I-related genes, $CD8^+$ T cell infiltration, and the production of the cytokines IFNG/IFN-γ (interferon gamma), TNF/TNF-α (tumor necrosis factor), and IL2 (interleukin 2) by $CD8^+$ T cells *in vivo*. Upregulation of NLRC5 expression also inhibits the negative role of LC3 in NLRC5-mediated MHC-I antigen presentation *in vivo*.

Thus, our study proposes the following: (1) NLRC5 serves as a target for immune evasion in endometrial cancer; (2) a negative correlation is found between NLRC5 and LC3 levels; (3) LC3 interacts with NLRC5 to inhibit the NLRC5-mediated MHC-I antigen presentation pathway (Figure 1). Our study suggests a potential immunotherapy strategy for

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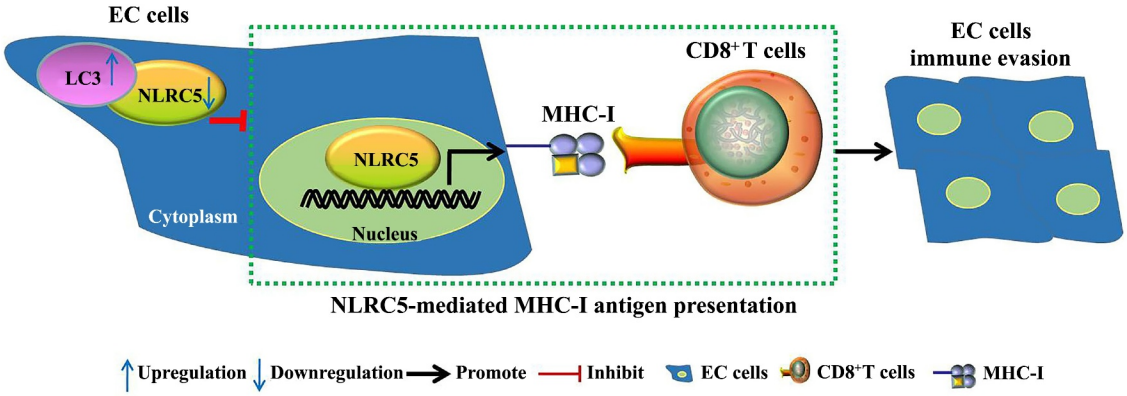

Figure 1. Model of EC development targeting NLRC5 by LC3 for immune evasion. LC3 is upregulated in EC patients. A high level of LC3 inhibits NLRC5 expression by interaction with NLRC5 in the cytoplasm of EC cells. NLRC5-mediated MHC-I antigen presentation in the nucleus of EC cells is crucial for killing of these cells. LC3 and NLRC5 interaction results in an impaired ability to elicit antitumor $CD8^+$ T cell responses in EC patients, which leads to immune evasion of EC cells and causes EC development.

the management of EC patients by inhibiting LC3 and promoting NLRC5. Consistent with our findings, recent studies have shown that inhibition of autophagy can obviously increase anti-tumor responses by promoting $CD8^+$ T cell infiltration into tumors. However, recent evidence has shown that NLRC5 can promote tumor cell proliferation, migration, and invasion, including in EC cells. Therefore, we are curious as to whether NLRC5 may also be a tumorigenic molecule and mediate resistance to immunotherapy, indicating the need for caution when promoting NLRC5 expression as adjuvant therapy in the immunotherapy for EC.

Therefore, we think the current findings are insufficient to translate to the clinic in EC, at least with regard to promoting NLRC5 expression as adjuvant therapy in the immunotherapy for EC. The following insights need to be investigated: First, although the combination of autophagy inhibitor with other therapies may be effective in cancer treatment, the efficiency of autophagy inhibitors in general is insufficient. Thus, the modulation of autophagy with drugs or adjuvants and the technologies for their delivery will continue to be the future focus in ongoing efforts to improve drug absorption and immunotherapeutic efficacy and to reduce the side effects of therapies. Second, LC3 antagonists should be under evaluation in clinical trials for their efficacy as adjuvants or drugs to activate the immune response in cancer patients. Third, it has been demonstrated that NLRC5 contributes to tumor progression by activating tumor cell-intrinsic signaling pathways. Thus, a tumor cell-intrinsic signaling pathways inhibitor may be able to attenuate the resistance to immunotherapy when promoting NLRC5 expression as adjuvant therapy. Through these considerations, we think that a dramatic increase in new autophagy- and NLRC5-based therapeutic options for cancer patients and a decline in clinical trial failures will be achieved.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Reference

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