



Identification of cancer-specific RNA modifications in extracellular vesicles and their pathological significance

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Abstract

RNA modifications play critical roles in cellular processes, and their dysregulation is associated with diseases, including cancer. Extracellular vesicles (EVs) carry various RNAs and can reflect the molecular state of their cells of origin, but detecting RNA modifications in EVs has been challenging due to their low RNA content. Using a proprietary ultra-high-performance liquid chromatography-tandem mass spectrometry system, we successfully detected 22 RNA modifications in EVs. In colorectal cancer (CRC), EVs exhibited reduced levels of N⁶-methyladenosine (m⁶A), which correlated with cancer recurrence. Increasing m⁶A levels via knockout of the demethylase Alkbh5 suppressed the tumor-promoting effects of CRC EVs. Mechanistically, CRC-derived EVs enhanced tumor necrosis factor α and interleukin-6 secretion by macrophages through Toll-like receptor 8 in an m⁶A-dependent manner, promoting cancer cell proliferation. RNA sequencing revealed higher levels of 5'-half-tRNA GlyGCC but lower m⁶A-modified 5'-half-GlyGCC in cancer EVs. These findings demonstrate that cancer-specific RNA modifications in EVs regulate immune cells and contribute to tumor progression.

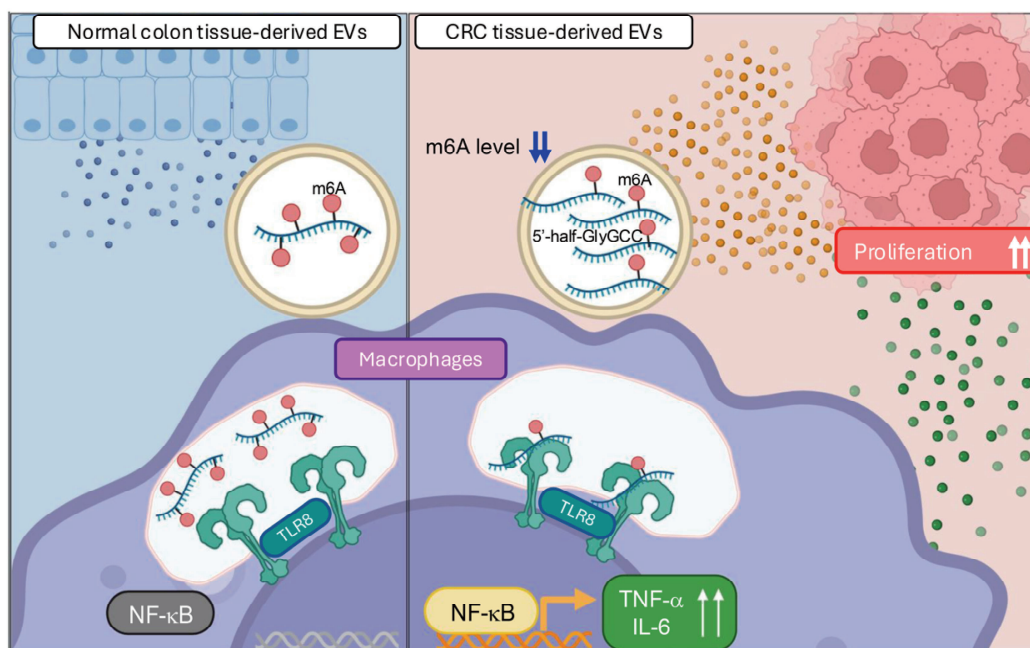
RNA on tumor development largely unclear. Colorectal cancer (CRC) is the third most common and second deadliest cancer worldwide, with tumor immune microenvironments, including immune cells and inflammation, playing a critical role in its progression. Systemic inflammation is associated with poor prognosis in 20–40% of CRC cases. Among RNA modifications, m⁶A (N⁶-methyladenosine), m⁵C (5-methylcytosine), and pseudouridine are common in mammalian RNA and can suppress host RNA recognition via Toll-like receptors (TLRs), which may distinguish self from non-self RNA. Extracellular vesicles (EVs) are small membrane-bound particles that mediate cell-to-cell communication. Using a high-sensitivity UHPLC-UniSpray-MS/MS system, we found that CRC-derived EVs contain high levels of 5'-half-tRNA GlyGCC with reduced m⁶A. This hypomethylated RNA activates macrophages via TLR8, increasing TNF- α and IL-6 secretion and promoting tumor growth, revealing a mechanism by which EV RNA modifications modulate the tumor microenvironment.

Significance of the research and Future perspective

This study revealed a novel mechanism by which RNA modifications in EVs released from cancer cells alter immune responses and promote tumor progression. This demonstrates that EVs not only transport RNA molecules but also actively regulate biological functions through the chemical properties of RNA modifications. Building on these findings, EV RNA modifications may serve as early diagnostic biomarkers for cancer, and targeting these modifications could enable the development of new immunotherapies.

Background & Results

RNA is a key molecule that regulates cellular functions, and its chemical modifications influence cancer progression and immune responses. Most previous studies have focused on enzymes controlling RNA modifications, leaving the direct impact of modified



Patent PCT/JP2024/032332, Japanese Patent Application No.2023-147093

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Keyword extracellular vesicles, cancer, RNA modifications