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HPV E7 Promotes Cisplatin Resistance in Cervical Cancer by Suppressing Necroptosis via Exosomal miR-21-5p

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Abstract. Cervical cancer, driven by high-risk human papillomavirus (HPV) infection, frequently develops resistance to cisplatin chemotherapy, thereby limiting treatment efficacy. Although the HPV oncoprotein E7 is known to interfere with cell death, its role in regulating necroptosis and chemoresistance remains unclear. This study reveals a novel mechanism by which HPV E7 promotes cisplatin resistance through exosomal miR-21-5p-mediated suppression of necroptosis. Using CRISPR/Cas9 to knockout miR-21-5p in HeLa cells, we demonstrate that miR-21-5p inhibition restores cisplatin sensitivity by reinstating necroptosis. In vivo xenograft models further confirm that miR-21-5p depletion reduces tumor growth and enhances chemosensitivity. These findings elucidate for the first time the role of the E7/exosomal miR-21-5p/MLKL signaling axis in cervical cancer chemoresistance, providing a potential therapeutic target for exosome-based strategies to overcome cisplatin resistance.

Keywords: necroptosis, miR-21-5p, cisplatin

1. Introduction

Cervical cancer, primarily driven by high-risk human papillomavirus (HPV) infection, remains a major global health burden despite advances in screening and vaccination [1]. Cisplatin-based chemotherapy is a cornerstone of treatment for advanced or recurrent disease, but the development of resistance severely limits its efficacy [2]. While HPV oncoproteins E6 and E7 are well-established drivers of carcinogenesis through p53 and Rb inactivation [3] , their role in chemotherapy resistance remains incompletely understood. Recent studies suggest that HPV E7 may modulate tumor microenvironment (TME) communication via exosomes [4], small extracellular vesicles that carry bioactive molecules such as microRNAs (miRNAs) [5]. Among these, miR-21-5p has emerged as a key regulator of drug resistance in multiple cancers [6], but its functional role in HPV-mediated cisplatin resistance,especially its involvement in necroptosis suppression, remains unexplored.

Necroptosis, a programmed form of necrotic cell death dependent on MLKL, serves as a critical fail-safe mechanism when apoptosis is impaired. Necroptosis induces RIPK1 autophosphorylation to activate RIPK3, or directly activates RIPK3 through Z-nucleic acids, finally leading to the activation of MLKL and cell lysis [7]. Given that HPV-infected cancers often exhibit apoptotic defects [8], necroptosis may represent an alternative therapeutic vulnerability. However, many tumors evade

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necroptosis through undefined mechanisms. It is reported that miR-21-5p is significantly upregulated in HPV-positive cervical cancer and enriched in exosomes from cisplatin-resistant patients [9,10]. Here, we demonstrate that HPV E7 enhances exosomal miR-21-5p secretion to suppress necroptosis, promoting cisplatin resistance.

Using patient-derived xenografts and in vitro models, we confirmed that miR-21-5p inhibition restores cisplatin sensitivity by reinstating necroptosis. This finding establishes a novel E7/exosomal miR-21-5p/MLKL axis in cervical cancer chemoresistance and highlight exosome-mediated miRNA transfer as a therapeutic target to overcome cisplatin resistance.

2. Rationale

HPV E7 protein promotes cisplatin resistance in cervical cancer by hijacking exosomal miR-21-5p in the tumor microenvironment to inhibit necroptosis. miR-21-5p may be significantly overexpressed in HPV-positive cervical cancer, with elevated levels detected in exosomes from chemotherapy-resistant patients. miR-21-5p is strongly associated with cell death evasion, and its inhibition could sensitize cancer cells to cisplatin. In HPV-infected cervical cancer, miR-21-5p directly targets the 3'UTR of RIPK1 or MLKL, suppressing the expression of key necroptosis executors. Furthermore, miR-21-5p propagates within the tumor microenvironment through exosome-mediated delivery, sustaining the resistant phenotype. Knockdown of miR-21-5p may restore RIPK1/MLKL-mediated necroptosis and overcome drug resistance.

3. Hypothesis

We predict that knock down of miR-21-5p plus cisplatin treatment in Hela cervical cancer cells leads to decreased viability, increased necroptosis, and decrease HeLa xenograft tumor size compared to cisplatin treatment without mir knock down.

4. Material and methods

4.1. Method 1 MTT assay

HPV-positive cervical cancer HeLa cells with miR-21-5p knockout were plated in 96-well plates at a density of 5 × 10^3 cells/well in 100 µL medium [11]. The negative control was HeLa cells with pbs. The positive control was Hela cell treated with TNF-α+z-VAD-FMK+SMAC mimetic (BV6). Cell viability was assessed using an MTT assay. Following an overnight incubation, cells were treated with 20 µL MTT (5 mg/mL) for 4 h at 37 °C. The formazan crystals were then solubilized with 100 µL of 0.1% acidified isopropanol, and the absorbance was measured at 570 nm [11].

4.2. Method 2 western blotting analysis

HPV-positive cervical cancer HeLa cells with miR-21-5p knockout were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, 10 U/mL penicillin, and 10 µg/mL streptomycin. The negative control was HeLa cells with pbs. The positive control was Hela cell treated with TNF-α+z-VAD-FMK+SMAC mimetic (BV6). Cell lysates were subjected to SDS-PAGE (10–15% gel), transferred to polyvinylidene difluoride membranes, and immunoblotted with the indicated primary and corresponding horseradish peroxidase-conjugated secondary antibodies after blocking. Signals were detected using an enhanced chemiluminescence kit and a chemiluminescence analyzer (Chemiscope600pro) [12].

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4.3. Method 3 in vivo tumor growth

Hela cells with miR-21-5p knockout (2 × 10^6 cells/mice) were subcutaneously injected to NSG mice to establish tumor xenograft model (n = 6). The negative control group was injected with Hela cells treated with pbs. And the positive contol group was injected with Hela cell treated with TNF-α+z-VAD-FMK+SMAC mimetic (BV6). Female mice (6-8 weeks old) weighing 18-22 g were used in the study. Tumor volumes were measured every week for four weeks, and mice were euthanized at forth week. Following weight measurement and fixation in 4% paraformaldehyde, tumor tissues were processed for subsequent detection. The study complied with all institutional guidelines for animal care and use.

4.4. Method 4 Crisper/Cas9 knockdown pre-miR-21

The human miR-21 gene is located on chromosome 17 (chr17:57,984,394-57,984,473). We designed sgRNAs targeting the pre-miR-21 precursor sequence of miR-21-5p to construct a CRISPR vector. At 48 hours post-transfection, cells were selected with puromycin (1–2 μg/mL) for 3–5 days, followed by isolation of monoclonal cell colonies.

4.5. Statistical analysis

Each experiment was repeated three times. Data were analyzed using SPSS 18.0 for Windows and are expressed as mean ± SD. Comparisons between two groups were made using Student's t-test, while multi-group comparisons were validated with Tukey's post-hoc test following ANOVA. A p-value of less than 0.05 was considered statistically significant [13].

5. Possible results

According to Table1, the combination of miR-21-5p knockout with cisplatin treatment leads to varying levels of support for the hypothesis that miR-21-5p enhances cisplatin's anti-tumor effect. Only when all three experimental outcomes are positive is the hypothesis fully supported. In contrast, the hypothesis is fully contradicted only when all outcomes are negative. All other mixed-result scenarios provide only partial support for the hypothesis.

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Table 1. Evaluation of the effect of miR-21-5p knockout combined with cisplatin treatment on cancer cell response and tumor growth

Combination Result # (CR#)	KO of miR-21-5p w/ cisplatin decreases viability by MTT compared to cisplatin alone	KO of miR-21-5p w/ cisplatin increases necroptosis by phospho-MLKL western blot compared to cisplatin alone	KO of miR-21-5p decreases tumor size by xenograft weight compared to cisplatin alone	Support of hypothesis
1	+	+	+	Full
2	+	-	+	Partial
3	+	+	-	Partial
4	-	+	+	Partial
5	-	-	+	Partial
6	-	+	-	Partial
7	+	-	-	Partial
8	-	-	-	Fully Contradicts

Table legend: "+" indicates that the measurement changes in the direction indicated similar to TNF-α+z-VAD-FMK+SMAC mimetic (BV6) opposite to DMSO control, "-" indicates the opposite.

CR1: KO of miR-21-5p leads to the decrease of viability and tumor size, and the increase of necroptosis compared to cisplatin alone.

CR2: KO of miR-21-5p leads to the decrease of viability and tumor size, and that necroptosis isn't increased compared to cisplatin alone.

CR3: KO of miR-21-5p leads to the decrease of viability and the increase of necroptosis, and that tumor size isn't decreased compared to cisplatin alone.

CR4: KO of miR-21-5p leads to the decrease of tumor size and the increase of necroptosis, and that viability isn't decreased compared to cisplatin alone.

CR5: KO of miR-21-5p leads to the decrease of tumor size, and that viability isn't decreased and necroptosis isn't increased compared to cisplatin alone.

CR6: KO of miR-21-5p leads to the increase of necroptosis, and that viability and tumor size aren't decreased compared to cisplatin alone.

CR7: KO of miR-21-5p leads to the decrease of viability, and that necroptosis isn't increased and tumor size isn't decreased compared to cisplatin alone.

CR8: KO of miR-21-5p doesn't lead to the decrease of viability or tumor size, nor does it increase necroptosis compared to cisplatin alone.

6. Discussion

CR1: This outcome fully supports our hypothesis that miR-21-5p promotes tumor cell growth by inhibiting necroptosis. I think about this result that miR-21-5p inhibits necroptosis in HeLa cells and thereby promotes tumor growth. The suppression of programmed cell death leads to abnormal proliferation of tumor cells. Knockdown of miR-21-5p can restore necroptosis and overcome drug resistance. Future work will further validate the pathway through which exosomes inhibit

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necroptosis. Deplete exosomes and examine the activation of key necroptosis-related molecules via Western blot. If necroptosis is not elevated upon knockout of this molecule, it would indicate that exosomes regulate necroptosis through this specific molecule.

CR2: In this case, the hypothesis is partially supported. I think about this result that miR-21-5p KO reduces cell viability and tumor volume but does not increase necroptosis. It may enhance cisplatin sensitivity through apoptosis rather than relying on necroptosis. Future studies will further investigate whether exosomes promote tumor growth by inhibiting apoptosis. Deplete exosomes and subsequently measure apoptosis levels by Caspase-3, Caspase-8, PARP-1, Bax, Bac, Bcl-2, Cytochrome c through WB, overall cell death by MTT, and tumor size.

CR3: In this case, the hypothesis is partially supported. I think about this result that the miR-21-5p inhibits necroptosis in HeLa cells, but in vivo effects may not be significant, possibly due to interference from CD8+ T cell in vivo. The necroptosis level of CD8+T cell may increase due to the overall high level of cell death in tumor environmont when miR-21-5p was knocked down. Future studies will exclude potential interference from decrease of CD8+ T cells. Conjugate the RIPK1 inhibitor Nec-1s with an anti-CD8 antibody to construct an antibody-drug conjugate (ADC).Use ADC to inhibit CD8+ T cell necroptosis, followed by repeating the in vivo experiments to observe whether tumor size decreases.

CR4: In this case, the hypothesis is partially supported. I think about this result that miR-21-5p KO reduces tumor volume and activates necroptosis, but does not decrease cell viability. The insufficient treatment time in vitro leads to activation of necroptosis, with increased expression of related molecules but incomplete cell lysis. Future studies will extend the digestion time to ensure complete cell lysis and then repeat the assay.

CR5: In this case, the hypothesis is partially supported. I think about this result that miR-21-5p KO reduces tumor volume without affecting cell viability or necroptosis. The reduction in tumor size may occur through mechanisms independent of cell death. miR-21-5p could influence the tumor microenvironment via exosomes rather than directly regulating cell death, such as by affecting blood supply. Future experiments will involve intratumoral injection of contrast agents followed by DCE-MRI to monitor changes in blood perfusion.

CR6: In this case, the hypothesis is partially supported. I think about this result that miR-21-5p KO solely activates necroptosis without affecting cell viability or tumor volume. The insufficient treatment time in vitro leads to activation of necroptosis, with increased expression of related molecules but incomplete cell lysis. Future studies will extend the digestion time to ensure complete cell lysis and then repeat the assay.

CR7: In this case, the hypothesis is partially supported. I think about this result that miR-21-5p KO only reduces cell viability without activating necroptosis or shrinking tumors. MiR-21-5p does not affect necroptosis. It can reduce cell death by increasing apoptosis. The in vivo effect of miR-21-5p KO is not significant, possibly due to decrease of CD8+ T cells. Future studies will further investigate whether exosomes promote tumor growth by inhibiting apoptosis. Deplete exosomes and subsequently measure apoptosis levels by Caspase-3, Caspase-8, PARP-1, Bax, Bac, Bcl-2, Cytochrome c through WB, overall cell death by MTT, and tumor size. Also conjugate the RIPK1 inhibitor Nec-1s with an anti-CD8 antibody to construct an antibody-drug conjugate (ADC).Use ADC to inhibit CD8+ T cell necroptosis, followed by repeating the in vivo experiments to observe whether tumor size decreases.

CR8: The result fully contradict the hypothesis. I think about this result that the sgRNA used may not effectively knockout miR-21-5p, or it might not influence cisplatin resistance in cervical cancer through regulating necroptosis. Future studies should first verify exosome levels after knock down.

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7. Conclusions

This study elucidates that HPV E7 promotes cisplatin resistance in cervical cancer through exosomal miR-21-5p-mediated suppression of necroptosis. By targeting miR-21-5p, we successfully restored cisplatin sensitivity in vitro and in vivo, underscoring the therapeutic potential of disrupting exosomal miRNA communication in the tumor microenvironment. These findings provide a foundation for developing novel strategies to combat cisplatin resistance in cervical cancer.

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