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Research Article

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Analysis of recombinant plasmid containing apoptin gene for apoptic activity

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Keywords

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Recombinant plasmid
Chicken anemia virus VP3
Expression
Apoptic activity

Abstract

The plasmid DNA containing the apoptin gene, derived from the chicken anemia virus, has been found to exhibit significant anti-tumor properties. This was evident through its action on HeLa cells in culture. Apoptin, a protein encoded by the apoptin gene, selectively induces apoptosis in tumor cells while sparing normal cells, making it a promising candidate for targeted cancer therapy. The study investigated the mechanisms underlying apoptin's tumor-specific cytotoxicity and its potential as a therapeutic agent. The results demonstrated that the introduction of the apoptin gene into HeLa cells led to the activation of apoptotic pathways, resulting in cell death. This research highlights the potential of apoptin as a novel approach in cancer treatment, offering a targeted and effective strategy against malignant cells.

Introduction

Apoptin, a small protein derived from the chicken anemia virus (CAV), has emerged as a promising candidate for cancer therapy due to its unique ability to selectively induce apoptosis in tumor cells while leaving normal cells unharmed. This tumor-specific property makes apoptin an attractive option for targeted cancer treatment, potentially minimizing the side effects associated with conventional therapies. The anti-tumor activity of apoptin has been demonstrated in various tumor cell lines and tumor models. For instance, Lee et al. (2007) reported that apoptin exhibited significant anti-tumor effects on canine mammary tumor cells, highlighting its potential for veterinary oncology. Furthermore, Natesan et al. (2006) showed that apoptin could effectively target and reduce Rous sarcoma virus-induced tumors, underscoring its broad applicability against different cancer types. The precise mechanisms by which apoptin induces apoptosis in tumor cells are not yet fully understood. However, it is believed that apoptin's selectivity is

linked to its ability to recognize and exploit specific vulnerabilities present in cancer cells, such as altered nuclear localization signals and dysregulated phosphorylation pathways. Despite the promising findings, the development of apoptin-based therapies faces challenges, including the need for efficient gene delivery systems and the optimization of treatment strategies to enhance its therapeutic efficacy. This study aims to contribute to the growing body of research on apoptin by investigating its anti-tumor effects on HeLa cells and elucidating the underlying molecular mechanisms, thereby advancing our understanding of its potential as a novel cancer therapeutic.

Materials and Methods

Vector

pVAX1-apoptin which was constructed using chicken anemia virus VP3 gene (Bhardwaj and Kothiyal, 2024) was used.

Cell culture

HeLa cell culture was grown in DMEM with 50µg/ml gentamycin and 10% fetal calf serum. flasks.

Transfection of Hela cells

Calcium phosphate DNA coprecipitate consisting of 100µl 2.5M $CaCl_2$, 20µlplasmid DNA and 80µldistilled waterin sterile microfuge tube was kept for 1 min and immediately transferred 20µl in each of 96 well microtiter plate. Then 100µl HeLa cells were added to each well keeping suitable controls without transfection. The plate was incubated at 37 ºC with 5% CO_2 tension for 72h (Rai *et al.*, 2020).

Immunoperoxidase Test

The cell culture medium was removedafter 72h of transfection,washed with PBS, fixed with chilled acetone at 4ºC for 1 min and air dried.Then Few drops of anti-apoptin mouse hyperimmune serum was added to monolayer and incubated for 1 h at37ºC. Then after washingwith PBS, put few drops of goat anti-mouse-HRP conjugate and kept at 37ºCfor 1 h, then washed with PBS, air dried and observed under microscope and photographed.

DNA fragmentation assay

After 48h of transfection,HeLa cellmonolayerwas trypsinised, and genomic DNA was isolated and the genomic DNA collected was analysed by 2% agarose gel electrophoresis.

Ethidium bromide- acridine orange staining

Staining was doneafter 48 h of transfection, using manufacturer'sprotocol.

Caspase 3 Detection

It was detected using Casp Glow^{TM} Fluorescein active caspase-3 staining kit (Biovision, USA)following manufacturer's method.

Annexin V binding Assays

Vibrant Apoptosis Assay Kit (Invitrogen, USA) as per manufacturer's protocol was used.

Results and Discussion

Expression Study

The IPT analysis of apoptin transfected cell culture revealed positive color reaction, while there was no color development in control cells (Fig.1-3).

Antitumor analysis of Apoptin

The DNA fragmentation assay revealed nucleosome laddering on agarose gel electrophoresis (Fig.4). Ethidium bromide acridine orange staining revealed nuclear condensation and fragmentation in apoptin-transfected cell culture but no such changes were seen in control cell culture (Fig.5-7). In caspase

detection assay, green fluorescence was seen in apoptin transfected cell culture but no changes in controls (Fig 8-10). Annexin-V-binding assay revealed bright green fluorescence in apoptin transfected cell culture while controls showed red fluorescence (Fig.11,12, 13).

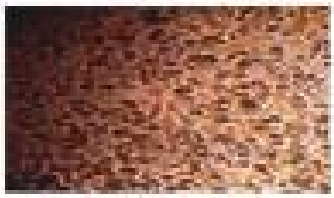

Figure 1. Apoptin transfected HeLa cells analysed by immunoperoxidase test showing expression.

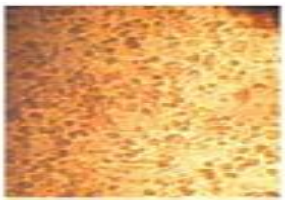

Figure 2.Mock-treated cell.

Figure 3.Vector treated cells.

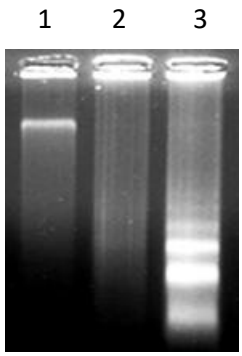

Figure 4: DNA laddering in HeLa cells
Lane 1: Mock transfected, 2: Vector transfected, 3: pVAX1-apoptin transfected.

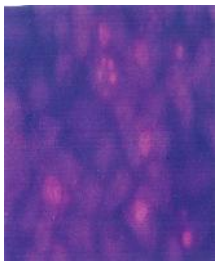

Figure 5. EtBr/acridine orange staining of pVAXI-apoptintransfected HeLa cells showing apoptotic effect.

Figure 6. EtBr/acridine orange staining of vector treated HeLa cell culture.

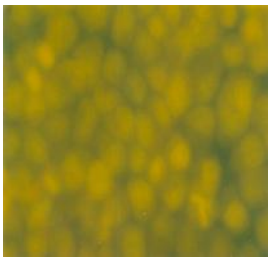

Figure 7. EtBr/acridine orange staining of healthy HeLa cells culture

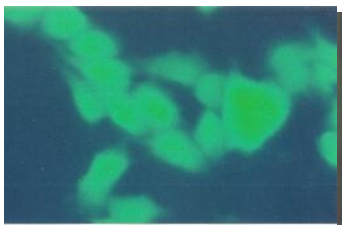

Figure 8.Caspase 3 detection of pVAXI-apoptintransfected HeLa cells showing positive cells.

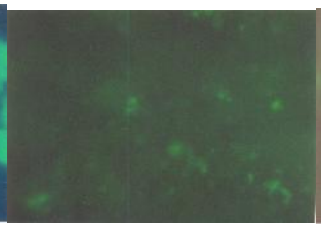

Figure 9.Caspase 3 detection of vector transfected HeLa cells.

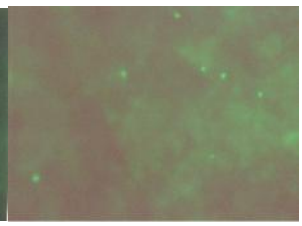

Figure 10.Caspase 3 detectionof mock transfected HeLa cells.

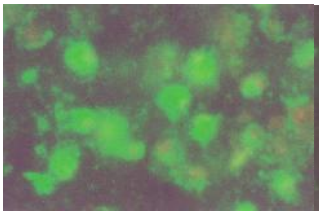

Figure 11. Annexin-V-binding assay of apoptintransfected HeLa cells showing apoptic cells.

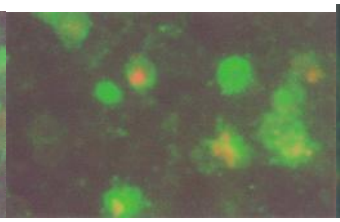

Figure 12. Annexin-V-binding assay of vector transfected HeLa cells.

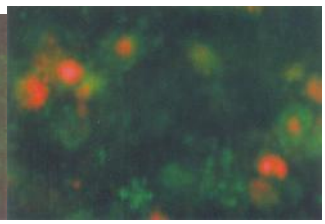

Figure 13. Annexin-V-binding assay of mock transfected HeLa cells.

Expression studies revealed apoptin expressed in cell culture system. The present study has demonstrated the apoptic activity of apoptin HeLa cell culture. Induction of apoptosis in HeLa cells revealed nucleosome laddering on agarose gel electrophoresis. Apoptic activity could also be demonstrated by EtBr-acridine orange, Caspase detection and Annexin-V- binding assay. Natesan *et al.* (2006) as well Lee*et al.* (2008) also performed EtBr-acridine orange staining to study apoptotic activity and found similar changes.Annexin-V-Binding assay and caspase detection assay done by us showed green fluorescence in apopticcells. Activation of caspase results in induction of apoptosis in human tumor cells and cell death is irreversible (Adams, 2003; Kumar, 2007; Denen *et al.*,2000).Zhan *et al.* (2012) showed aqnti-tumor activity of apoptin.

Conclusion

In conclusion, this study demonstrated that apoptin, a protein derived from the chicken anemia virus, exhibits significant anti-tumor activity. When introduced into HeLa cells, a well-established model for studying cancer, apoptin selectively induced cell death through apoptosis, the body's natural process for removing damaged or unwanted cells. This finding is consistent with previous research showing apoptin's ability to target and eliminate cancer cells in various contexts, including canine mammary tumor cells and Rous sarcoma virus-induced tumors (Lee et al., 2007; Natesan et al., 2006).

The results of this study contribute to the growing body of evidence supporting apoptin's potential as a novel cancer therapeutic. Its unique ability to distinguish between cancer cells and healthy cells makes it an attractive candidate for targeted therapy, as it may help to minimize the side effects often associated with traditional cancer treatments. By exploiting specific vulnerabilities present in cancer cells, apoptin could offer a more precise and effective approach to treating malignancies.

However, several challenges must be addressed before apoptin-based therapies can become a clinical reality. One major hurdle is the development of efficient and safe gene delivery systems to ensure that the apoptin gene is effectively introduced into cancer cells. Additionally, the optimal treatment strategies, such as the best timing and dosage for apoptin administration, need to be determined to maximize its therapeutic benefits.

Furthermore, while this study focused on HeLa cells, future research should investigate apoptin's anti-tumor effects in other cancer types and in more complex models, such as animal studies and eventually human clinical trials. These investigations will help to establish the broad applicability of apoptin as a cancer treatment and facilitate its translation into clinical practice.

In addition to its therapeutic potential, studying apoptin can also provide valuable insights into the fundamental differences between cancer cells and normal cells. By understanding how apoptin selectively targets cancer cells, we may uncover new biomarkers and mechanisms that can be exploited for the development of other targeted therapies.

In summary, this study highlights the promising anti-tumor activity of apoptin and underscores the need for continued research to unlock its full potential as a cancer therapeutic. By addressing the current challenges and building on the existing knowledge, we can move closer to harnessing the power of apoptin for the safe and effective treatment of cancer.

This expanded conclusion summarizes the key findings, discusses their implications, acknowledges challenges, and suggests future research directions.

Conflict of interest

The authors declare that they have no conflict of interest.

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