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Iridin Attenuates the Migration and Attachment Independent Growth of Lung Adenocarcinoma Cells

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Abstract: ***Lung adenocarcinoma is the leading cause of lung cancer related deaths worldwide. The low survival rates associated with this cancer are being attributed to metastasis and recurrence which contribute to the failure of treatment. In this study we studied the ability of Iridin, anisoflavone isolated from Iris Kashmirianato ablate the metastatic progression in human lung adenocarcinoma. Human lung adenocarcinoma cells, A549 were treated with various concentrations of Iridin for 24 h to evaluate their effect on their proliferation by MTT assay. Only those concentrations which showed least effect on the proliferation of A549 cells were used to examine their effect on migration and attachment independence. The effect of such concentrations on the migration and attachment independence of A549 cells was determined by wound healing and soft agar colony formation assays respectively. The results demonstrated that treatment with Iridinled to significant inhibition in the migration as well as colony formation in lung adenocarcinoma cells. Taken together, these findings present a preliminary account of anti-metastatic activity of Iridin which warrants further investigation to decipher its mechanistic action.***
Keywords: Lung adenocarcinoma; Iridin; Migration; Attachment independence*.*

I. INTRODUCTION

Lung cancer is one of the leading causes of cancer-related deaths globally. Broadly lung cancer is divided into two sub-types i.e., Small cell lung cancer and non-small cell lung cancer. Non-small cell lung cancer (NSCLC) accounts for 85% of the total lung cancer cases and includes two major histological sub-types, adenocarcinoma and squamous cell carcinoma [1]. Lung adenocarcinoma is most common form and constitutes about 50% of NSCLC [2].

Despite the significant advances in lung cancer diagnosis and treatment, the prognosis of these patients remains dismal, with 5 years survival rates less than 10% [3]. There occurs frequent relapse with metastasis even in the patients who have undergone complete surgical resection. Many factors are considered to be responsible for abysmal success in lung cancer treatment. Chemotherapy continues to remain a preferred therapeutic strategy for cancer treatment, but it is accompanied by a wide variety of side effects. Furthermore owing to drug resistance exhibited by most of the tumors, it fails to eliminate all the tumor cells eventually resulting in metastasis [4].In the context of cancer progression, migration and attachment independence constitute the important factors that must be taken into account while designing a therapeutic strategy [5].

Therefore, these processes serve as a promising target of therapeutic intervention to overcomes the metastatic progression in tumors. Since times immemorial natural products have been used to treat various disorders and ailments. They have proved to be effective chemo preventive agents and saferanti-cancer treatments [6,7]in comparison to conventional anti-cancer therapies (8). Iridin is an isoflavanone isolated from various Iris species and has traditionally been used for the treatment of liver problems and also to expel intestinal parasites. [9]. A few studies have reported the cytotoxic activity of Iridin on human cancer cells [10].However, there areno reports that show the effect of Iridin on the migration and attachment independence of human lung adenocarcinoma cells. In this report we show that Iridin effectively limits the proliferation of A549, lung adenocarcinoma cells with concomitant inhibition of their migration and attachment independence.

II. MATERIALS AND METHODS

A. Materials

DMEM and FBS were procured from HyClone, USA. Penstrip was obtained from Invitrogen, USA. Iridin was isolated from Iris Kashmiriana(11). DMSO and MTT were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

B. Cell culture

Human lung adenocarcinoma cellsA549was a kind gift from Dr. AyubQadri, National Institute of Immunology. Cells were cultured in DMEM, supplemented with 10% fetal bovine serum, and antibiotics (1% penstrip) at 37 °C in 5% CO2 humidified incubator. The medium was changed regularly and the cells were sub-cultured every 3-4 days.

C. Cell Proliferation Assay

MTT assay was used to check the viability of cells. This assay is based on the conversion of MTT to formazan by the action of mitochondrial dehydrogenises present in the live cells. Cells were seeded into the wells of a 96 well plate and allowed to grow overnight. DMSO was used as solvent (vehicle) to dissolve the Iridin, which was added to cells at various concentrations (5- 40µM) for 24 h. After treatment, MTT solution was added to each well followed by 2 h incubation at 37°C in dark. Then the MTT solution was removed and equal volume of DMSO was added to cells to solubilize the formazan. Finally the absorbance was measured at 590 nm using micro plate reader (Biotek, USA). Following formula was used to measure the inhibition of cell proliferation.
Percentage proliferation inhibition = (1 – OD sample/OD control) × 100%.

D. Cell migration Assay

0.2 × 106A549 cells were platted in each well of 24-well plate and the cells were allowed to grow to80-90% confluence. Then the medium was removed and the wound was created using a 100 µl pipette tip. Then the washings were carried out using PBS to remove the floating cells. This was followed by treatment with sub-toxic concentrations of Iridin for 24 h. Photographs were taken before (0 h) and after treatment at 24 h. Wound healing was calculated as the percentage of the initial wound before treatment to the total wound closure at 24 h after treatment. The assay was performed three independent times.

E.Soft agar colony formation assay (Clonogenic assay)

Anchorage independent growth in soft agar was used to evaluate the effect of Iridin on the tumorigenic potential of A549 cells. The soft agar assay was performed in 6-well plates containing two layers of Agar. The bottom layer consisted of 0.8% agar in 1 ml of DMEM supplemented with 10% FBS. Cancer cells (1x104/well) were placed in the top layer containing 0.4% agar in the same medium as the bottom. Cells were cultured for 2 weeks under different concentrations of Iridin and colonies were photographed and counted per four fields under a microscope.

III. RESULTS

A. Iridin inhibits the proliferation of A549 cells

A549 human lung adenocarcinoma cells were cultured in presence of increasing concentrations of Iridin (5-40 µM) for 24 h in order evaluate the effect of Iridin on their proliferation. The effect of Iridin on the proliferation of these cells was evaluated after 24 h of exposure to varying concentrations of Iridin by MTT assay. It was observed that exposure of A549 cells to different concentrations of Iridinled to a significant growth inhibition. The rate of proliferative of A549 cells decreased in presence of Iridin in a dose-dependent manner with IC_{50} value of 37 µM. At lower concentrations of 5 µM, 10 µM and 15µM least cytotoxic effects were observed but there was significant inhibition in proliferation of A549 cells at higher concentrations of 25 µM, 30 µM, 35 µM and 40 µM (Fig. 1). These observations therefore led us to use least cytotoxic concentrations of Iridin for subsequent experiments.

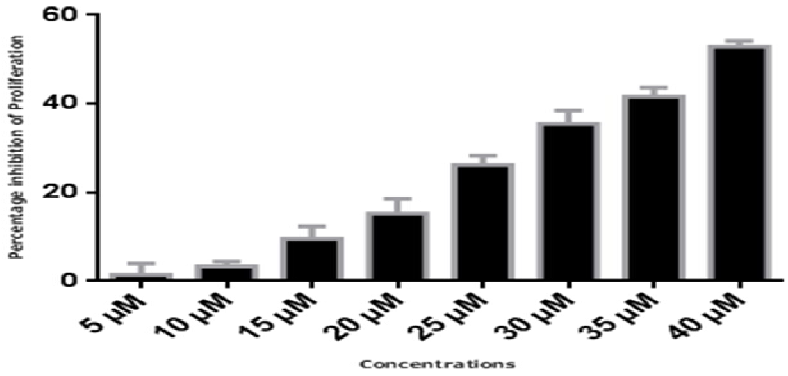

Fig 1.*Iridin* inhibits the proliferation of A549 cells. Cells were treated with increasing concentrations of *Iridin* (5, 10, 15, 20, 25, 30, 35 and 40 µM) for 24h followed by MTT assay.

B. Iridin ablates the migration of A549 cells

In order to evaluate the effect of Iridin on the migration of A549 cells, wound healing assay was carried out. The cells were grown to confluence and the wound was created using a pipette tip. Then the cells were treated with sub-toxic concentrations of Iridin for 24 h. The distance covered by the cells to heal the wound compared to initial time point when the wound was created was measured and the results were expressed as percentage wound healing. It was observed that Iridin significantly led to the inhibition of migratory abilities of A549 cells in comparison to control. The effect was found to be dose dependent with 15µMconcentration being the best (Figure 2). The above findings depict that treatment with Iridin significantly inhibited closure of the wound, thus pointing at the ability of Iridin to overcome the migration A549 cells.

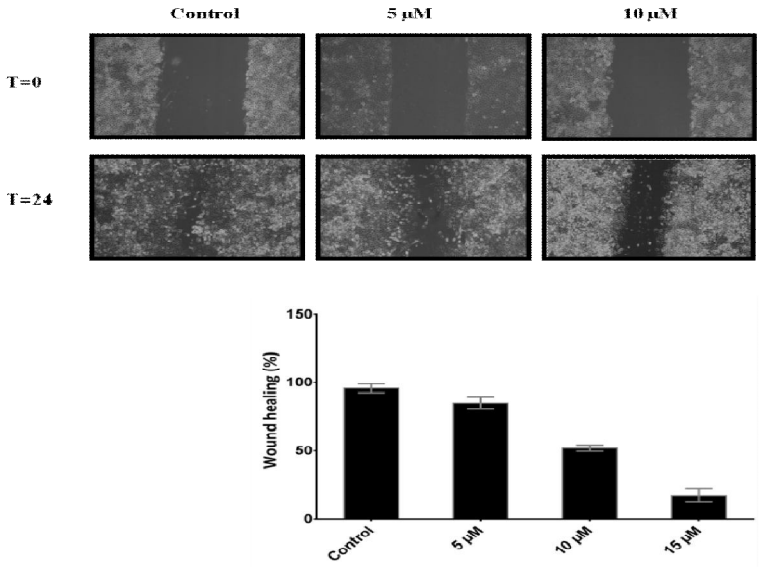

Fig 2.Iridin inhibits the migration of A549 cells. Cells were either treated with sub-lethal concentrations of Iridin (5 µM, 10 µM and 15 µM) or DMSO (control) and the wound healing was observed after 24 h.

C. Iridin limits the attachment independent growth of A549 cells

Anchorage-independent growth, the ability of a cell to proliferate without attachment to, or spreading onto a substratum is one of the hallmarks of transformation and the most accurate in vitro indication of tumorigenicity. To assess whether Iridin inhibits the tumorigenic potential of A549 cells, soft agar colony formation assay was performed wherein A549 cells were grown under attachment independent conditions in presence of different sub-lethal concentrations (5µM- 15 µM) of Iridin for two weeks. It was observed that there was dose dependent decrease in the formation of colonies by A549 cells when treated with Iridin(Figure 3).

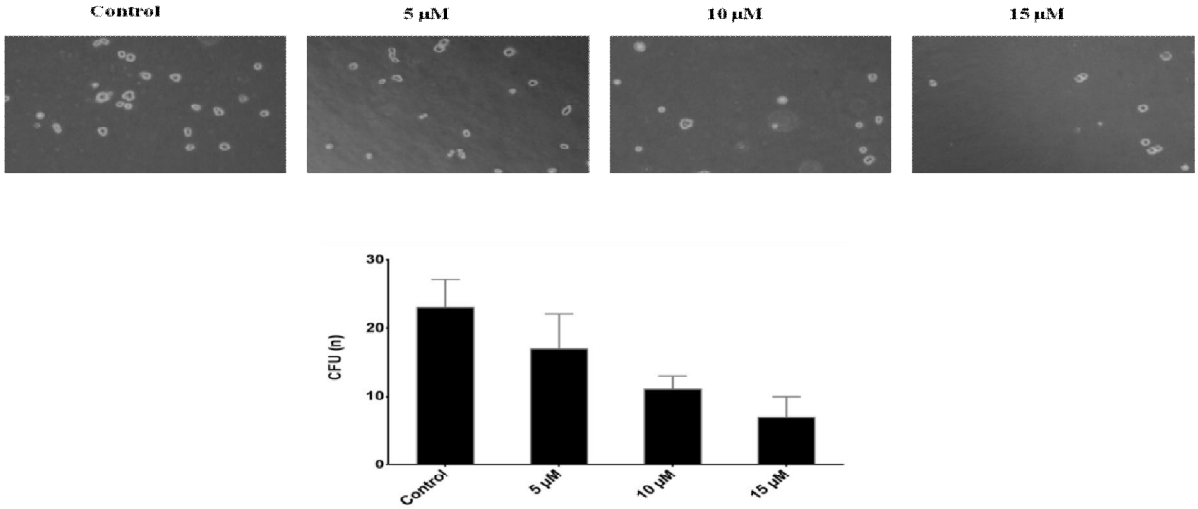

Fig 3.Iridin limits the colony formation of A549 cells. Cells were either treated with sub-lethal concentrations of Iridin (5 µM, 10 µM and 15 µM) or DMSO (control) and the efficiency of colony formation was observed after 2 weeks.

IV. DISCUSSION

Despite progress made in understanding and the treatment modalities of cancer, Lung adenocarcinoma continues to remain the leading cause of lung cancer related deaths [12]. This is mostly attributed to the metastatic tendencies and resistance to various treatment regimens such as radiotherapy, chemotherapy and targeted therapy exhibited by lung adenocarcinoma. Tumor metastasis is a complex process in which tumor cells dissociate and migrate from the primary tumor site, travel through circulatory system, braving all the odds and form metastatic colonies at a distant site. Because lung cancer is often diagnosed at advanced stages and fails to respond to therapy, novel strategies that focus on minimizing lung cancer metastasis are urgently needed.

Natural products represent the important chemo preventive agents and are increasingly being used for anticancer activity [13]. Iridin anisoflavan one isolated from the various species of Iris plant has been traditionally used to cure various ailments [10]. However there are no reports that have evaluated the anti-metastasis effect of Iridin in detail. Therefore the present study was carried out to examine the effect of Iridin in limiting the migration and attachment independence of human lung adenocarcinoma cell. The present study is the first report of its kind to show the antimeta static effect of Iridin on lung adenocarcinoma cells. We show that Iridin was highly potent in overcoming the proliferative index of A549 cells. The effect of Iridin on the growth of A549 cells was observed to be dose dependent with IC50 value of 37 µM. It was observed that treatment led to the disruption of metastatic process in A549cells as accessed by migration and colony formation assays. The results pointed out that Iridin could efficiently overcome the migration and colony formation of A549 cells in a dose dependent manner. The present finding provides preliminary evidence that Iridinimpedes the metastatic process in lung adenocarcinoma and thus lays impetus on working out the underlying mechanism.

V. CONCLUSION

In conclusion this study reports the *in-vitro* anti-metastatic activity of Iridin on lung adenocarcinoma cells and represents a lead for further studies to substantiate the use of this bioactive compound.

VI. ACKNOWLEDGEMENT

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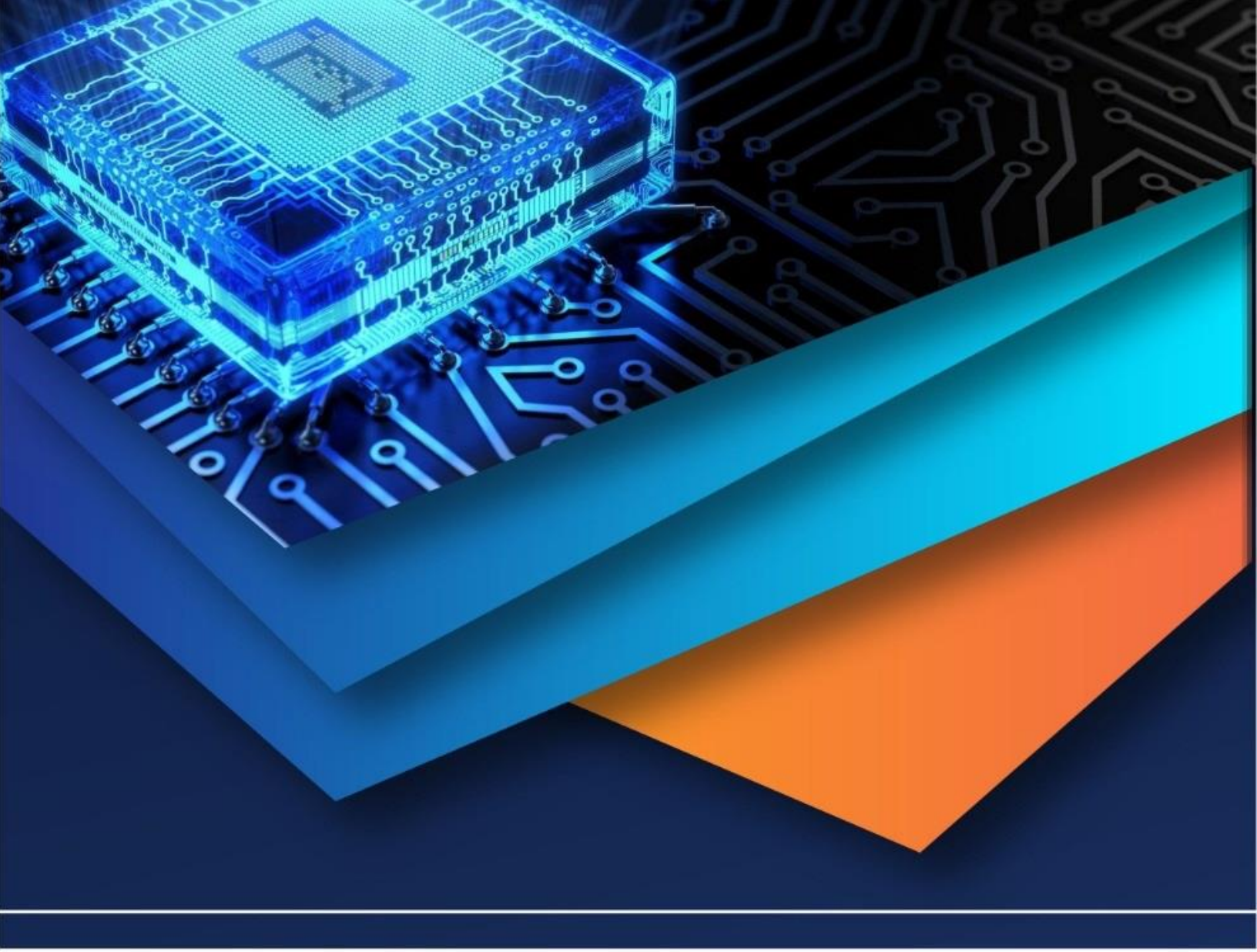

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