5-Azacytidine treatment determines the epigenetic reprogramming of lung cancer models followed by a significant anti-cancer activity

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**5-Aza effects upon A549 cell viability and cell cycle**

We evaluated the effects upon apoptosis and cell cycle through flow cytometry as response to irradiation, two repITIVE doses of 5-Aza and 5-Ara in combination with irradiation. Data showed that 5-Aza induces cell apoptosis and also secures and is stopping the cell cycle by blocking the cell in the G2 phase independent of the irradiation.

**5-Aza inhibits the clonogenic capacity of lung cancer cells**

We next evaluated the clonogenic and invasion capacity of treated cells compared to control. The results show almost complete loss of colony forming capacity and significantly inhibited invasion in the case of 5-Aza treatment.

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**Introduction**

At worldwide level lung cancer remains the leading malignant pathology in terms of incidence (11.6% of the total diagnosed cases for both sexes combined), but also for mortality rates (14.4% of the total mortality cases for both sexes combined) (Global cancer statistics, 2018). Although, the American Cancer Society recommends intensive screening programs in order to diagnose the malignant tumor in early stages and obtain a good treatment evolution, the overall survival is still at 19% for both sexes and pathological subtypes combined (American Cancer Society, 2020). Even the ones diagnosed in their early stages are prone to develop acquired resistance to the treatment (Hofhun et al., Nat Rev Cancer, 2013).

The installation and development of lung cancer brings together genetic, epigenetic and environmental factors that contribute to installation of aberrant signaling networks with overexpressed oncogenes and downregulated tumor suppressor ones combined with mutated genes with modified/silenced function. The discovery of such aberrations is translated in numerous funds invested in development of targeted therapeutic agents that match such molecular alternations at gene or protein level (e.g. EGFR, ALK, BRAF, RET, MET inhibitors) (American Cancer Society, 2020).

However, the main issue is that 50% of Non-Small Cell Lung Cancer (NSCLC) cases (NSCLC accounts for 80% of lung cancer cases) have no identified targetable mutations and, in the case of those who are positive for specific mutations, the development of therapy resistance intervenes most of the times (Ansari et al., Transl Lung Cancer Res, 2016).

**Study objective**

In the current context of lung cancer heterogeneity, epigenetics could become an advantageous field of study. These types of modifications are reversible and are now considered an individual cancer hallmark. The most recognized epigenetic alteration consists in DNA methylation with impact on gene silencing and chromatin structure. DNA methyltransferases (DNMTs) mediate this process through covalent addition of methyl derivatives to a cytosine, yielding 5-methylcytosine (5mC). Lung cancer is no exception, where epigenetic modifications are standing at the base of tumor instability and development through inhibition of tumor suppressor genes and dysregulation of oncogenes (Shi et al., J of Oncol, 2019).

Although most probably these changes are dynamic and not constant the same between patients or lung cancer stages, the malignant cell will not invest the energy in a process that is not in its favor. Therefore, demethylatig strategies may circumvent issues like tumor heterogeneity and patient inviability, reversing the global DNA methylation that sustains the malignant processes.

**Materials and methods**

The present study investigated the repositioning of 5-Azacytidine (in combination or not with irradiation) for the treatment of lung cancer in both in vitro and in vivo models.

**Cell lines and treatment scheme**

A549 and A549-LacZ cell culture were cultivated in F-12K medium supplemented with 10% fetal bovine serum (FBS). All cell lines were maintained at 37 °C with 5% CO2. Cells were treated with 5-Azacytidine (5-Aza) (4-Amino-1-

**In vitro functional tests**

To investigate the therapeutic role of 5-Aza in A549 cell line, we performed the following functional tests: cell viability assay, flow cytometry for apoptosis and cell cycle, transwell assay and wound healing assay, colony formation and spherical formation.

**In vivo evaluation**

Eight week-old female athymic nude were included in the study. All experimental protocols were approved by the Ethics Committee of Iuliu Hatieganu University of Medicine and Pharmacy and were conducted in accordance with the EU Directive 65/101. We validated the therapeutic effect of 5-Aza in lung cancer by developing spontaneous and orthotopic mouse models. The mice were injected subcutaneously in the right flank with 2×106 A549 cells previously treated according to treatment scheme mentioned above. Tumor measurements and animals weigthing were done once in 3 days for 30 days. In the case of orthotopic model, mice were injected into the right lung with 2×106 A549-LacZ cells through an open surgery surgery procedure. The animals were divided in treatment group and control group (two animals/group): the treatment group received concomitantly for 5-Aza (1mg/kg) dissolved in 150 µl of saline solution (intraperitoneally administration) and the control group received the same treatment scheme but only with 150 of µl of saline solution. The efficiency of the treatment was monitored with the IVIS Imaging System once a week and at the end of the experiment.

**Results**

The distribution of Under-expressed Genes on Chromosomes

Lung cancer is mainly based on mechanisms of downregulation with 313 underexpressed genes (green) and 1111 overexpressed ones – data not shown distributed among chromosomes.

Moreover, data shows that there are 4114 hyper-methylated CpGs and only 120 hypo-methylated ones (data not shown) in UAD compared to paired normal tissues.

**Methylation profile of lung cancer**

The distribution of Hyper-methylated CpGs on Chromosomes (Number of CpGs: 4114)

**References**


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