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ABSTRACT OF DISSERTATION

N^6 -furfuryloadenosine (kinetin riboside, KR) is an adenosine derivative that inhibits tumor cell proliferation. Its activity is dependent on adenosine kinase, which determines its toxicity.

To test whether RK could be involved in the intracellular metabolic flux of purines and thus interfere with the function of purine biosynthesis enzymes, I used a stable isotope. Labeled KR was metabolized by tumor cells, leading to the production of metabolites. Using various complementary cellular assays, I traced the pathway of intermediate metabolites resulting from enzymatic transformation of [^{13}C]KR in hepatocellular carcinoma cells (HepG2 cell line) and demonstrated the presence of numerous modifications in DNA and RNA. Such detailed studies revealed the complexity of kinetin riboside action in cancer cells, causing metabolic disturbances, purinosome association and ultimately induction of cell death. My results indicate that all three pathways (*de novo*, salvage and catabolic) are involved in the metabolism of purine derivatives and regulation of their biosynthesis and transformation in cancer cells, complementing each other by providing intermediate metabolites for subsequent synthesis.

I confirmed that kinetin riboside in cancer cells exhibits a complex mechanism of action, affecting multiple levels of cell organization. Its presence disrupts cancer cell metabolism, causing energy, oxidative imbalance, inhibiting cancer cell proliferation.

Therapies based on small-molecule compounds have enabled new insights into cancer treatment with respect to mitochondrial dysfunction, such as reduction of mitochondrial membrane potential, induction of oxidative stress, or effects on their morphology. I have shown that KR is a compound that selectively affects molecular pathways crucial for cell growth and apoptosis by interfering with mitochondrial function, and thus may be a potential mitotoxic agent. Cancer cell metabolism relies primarily on the Crabtree effect, which involves glucose-induced inhibition of cellular respiration and thus oxidative phosphorylation (OXPHOS), supporting cancer cell survival under metabolic stress. The simplest way to circumvent this phenomenon was to replace glucose with galactose in the culture medium. As a consequence, cells became more sensitive to mitochondrial perturbations induced by the mitotoxic factor. In the present study, I examined the effect of KR on mitochondrial function in HepG2 cells forced to rely mainly on OXPHOS.

I showed that KR in galactose environment is a stronger inducer of apoptosis, decreases mitochondrial membrane potential, reduces glutathione levels, depletes cellular ATP and induces production of reactive oxygen species (ROS) in OXPHOS state, leading to loss of cell viability.

The final step of my research was to demonstrate the effect of KR and its derivatives on the redox status of glioblastoma multiforme cells.

Anticancer therapies based on small-molecule compounds, by impairing oxidative balance, could also represent a novel therapeutic approach for the treatment of glioblastoma multiforme (GBM). Kinetin riboside and newly designed derivatives (8-azaKR, 7-deazaKR) selectively affect molecular pathways crucial for cell growth by disrupting the redox status of tumor cells. This makes these compounds a potential alternative for oxidative therapy of GBM. Elevated basal levels of reactive oxygen species (ROS) in GBM support cancer cell survival and induce drug resistance. The simplest way to induce cell death is to reach the redox threshold and bypass antioxidant defense mechanisms. Consequently, cells become more sensitive to oxidative stress (OS) induced by exogenous factors. In the present study, I investigated the effect of KR and its derivatives on the redox status of T98G cells in 2D and 3D cultures. Using spheroids of T98G cells allowed me to select one derivative, 7-deazaKR, with comparable antitumor activity to KR. Both compounds induced the formation of ROS and genotoxic OS, causing lipid peroxidation and leading to apoptosis. These results indicate that KR and 7-deazaKR modulate the cellular redox environment of T98G cells, and the sensitivity of these cells depends on the efficiency of antioxidant mechanisms.