Advances on photodynamic therapy through new pyridine-fused diphenylchlorins as photosensitizers for melanoma treatment

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Aim: Assessment of cytotoxicity of four new photosensitizers intended for photodynamic therapy (PDT) against melanoma cells (A375 cells).

Introduction: Melanoma is the rarest form of skin cancer. PDT combines a photosensitizer with light culminating in the production of reactive oxygen species leading to cellular death. A new type of stable 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused tetraphenylchlorins1, proved to be very active as photodynamic agents. Thus, looking for a new generation photosensitizers with optimized properties for PDT we synthesized new diphenylchlorins.

Methods: The human melanoma cell line A375 was seeded in 48 well plates. The photosensitizers NAMP103A, NAMP103B (the tetraphenylchlorins monooester), NAMP263A and NAMP263B (the tetraphenylchlorins alcohol) were administered ranging 5 nM to 10 μM. Irradiation was performed after 24 h (λ < 560 nm). MTT and SRB assays as well as flow-cytometry were performed 24 h after the PDT.

Results: MTT assay results allowed to obtain dose-response curves and to calculate the concentration that inhibits the cultures (IC50). Phototoxicity (10J) was dependent on the chlorines concentration. Moreover, NAMP263B was significantly more cytotoxic than NAMP103A (p = 0.037) and NAMP103B (p = 0.042). From SRB assay we verified that with a 125 nM concentration the NAMP103A, NAMP103B, NAMP263A and NAMP263B produce a cellular viability of 36.9%; 33.2%; 18.3% and 18.8%, respectively. Flow cytometry studies confirmed the decrease of viability associated with cell death by apoptosis and necrosis. Loss of mitochondrial membrane potential, apoptosis hallmark, was also observed. An imbalance of ROS, namely superoxide anion and peroxides, was observed for all photosensitizers studied with an exhaustion of antioxidative intracellular defenses (GSH). At low PS concentrations (5 nM), metabolic activity was variable with light energy (5 J, 10 J and 20 J) with lower values for higher fluence. Dark toxicity studies revealed photosensitizer dependence of irradiation.

Conclusion: We hereby conclude that the photosensitizers are indeed very promising, which rouses plans for following proceedings to verify in vivo outcome.

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Angiogenesis and inflammation at the crossroads between diabetes and cancer

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Aim: To study fibrosis, angiogenesis, oxidative stress and inflammation markers in diabetic and non-diabetic patients with gastric cancer (GC).

Introduction: Type 2 Diabetes mellitus (DM2) is a major health problem, with 415 million people diagnosed worldwide. Evidence regarding its association with various types of cancer has been reported, including GC. Some hypotheses have been suggested to explain how DM2 could enhance the risk of cancer development, such as hyperglycemia, hyperinsulinemia, oxidative stress, vascular disturbances and a chronic low inflammation state.

Gastric cancer (GC) is the fifth most common cancer worldwide and ranks as the third leading cause of cancer-related death. GC is frequently associated with infection by Helicobacter pylori and inflammation plays a central role in the carcinogenic process. Such chronic inflammatory state, linked with angiogenesis imbalance, oxidative stress and metabolic signaling, suggests that also DM2 might be a major risk factor in initiation and progression of GC, demanding further investigation.

Methods: A series of GC from DM2 (n = 22) and nonDM2 (n = 21) patients were studied. Immunohistochemistry (IHC) using antibodies against CD31 and 3-Nitrotyrosine was performed, to assess density of vessels and oxidative stress status. Histochemical staining with Sirius red was performed to determine the percentage of fibrosis in the tumor and non-neoplastic adjacent mucosa. Based on assessment of tumor inflammatory cell infiltrate and tumor stroma...
percentage, a semi-quantitative evaluation of Glasgow Microenvironment Score was performed. Also, Glasgow Prognostic Score, that is widely known as a systemic inflammatory-based marker, was determined for each patient.²

**Results:** Diabetic patients presented a significant higher glycaemia than the control patients (190.1 ± 13.6 mg/dL vs 98.2 ± 3.6 mg/dL, p < 0.001, respectively). Decreased survival rates were observed in diabetic patients (S11.5 vs 916.0, p = ns). Tumours exhibited increased fibrosis relatively to the adjacent mucosa in both groups and diabetic patients (N: 9.362 ± 1.337; T: 12.29 ± 1.407) presented higher fibrosis levels than the non-diabetic patients (N: 7.165 ± 1.017; T: 10.97 ± 1.076).

**Conclusion:** Expected results: Identifying the distinct features that characterize GC of DM2 patients compared to non-diabetic patients (namely fibrosis, angiogenesis, inflammation, and oxidative stress biomarkers) will enable to study this subset of GC patients and unravel key mechanisms behind the relationship between DM2 and GC.

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**Circulating EVs for AML minimal residual disease biomarkers detection**

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**Aim:** We propose to evaluate the feasibility of a peripheral blood EV-based liquid biopsy method for AML disease monitoring in real time with molecular precision.

**Introduction:** Acute myeloid leukemia (AML) is a hematopoietic stem cell disorder with high mortality rate mainly due to the high frequency of post-treatment relapse. Minimal residual disease (MRD) determination in AML patients receiving treatment is useful to assess chemotherapy response and predict relapse. One approach to upgrade the current invasive MRD monitoring (traditionally based on bone marrow aspirates/biopsies) is to use methods that identify cancer-associated biomarkers in patients’ blood. Recently, extracellular vesicles (EVs) have been increasingly recognized as a potential source of biomarkers, since the levels of EVs are markedly increased in cancer patients’ blood and those EVs potentially carry molecular signatures associated with specific cancer phenotypes.

**Methods:** The profile of EVs isolated from AML patients’ blood plasma collected from paired AML diagnostic and complete remission samples is being compared and correlated with clinical data. A size-exclusion chromatography (SEC) method was optimized to isolate the plasmatic EVs. The EVs profile is then characterized according to their size, plasmatic concentration, morphology and protein content.

**Results:** EVs with decreasing size were successfully isolated between SEC fractions 3 to 6, with a size ranging from 300 nm to 30 nm, respectively. Fraction 7 presented the smaller EVs, although mixed with some plasmatic protein contaminants. The expression of EV markers such as CD63, HSP70 or Syntenin-1 was confirmed and allow to distinguish EV subpopulations between fractions 3 to 7. The expression of leukemia-specific markers is currently being studied in the EVs isolated from the paired AML blood samples.

**Conclusion:** The presented EV-based liquid biopsy proposed method for AML monitoring could unravel biomarkers for diagnostic and prognostic purposes in AML patients.