M U N I

COMMENTARY TO HABILITATION THESIS[1]

Recent decades have seen major breakthroughs in cell death research. The concept of regulated, genetically driven lytic processes such as necroptosis, pyroptosis, and ferroptosis has challenged the outdated view of necrosis as purely unprogrammed. Despite extensive understanding of apoptosis, alternative death pathways remain underexplored, especially in head and neck cancers, where molecular heterogeneity affects treatment outcomes.

Advances in light microscopy, particularly quantitative phase imaging (QPI), have revolutionized cell biology. As all types of cell death involve mass changes, apoptotic disintegration or membrane alterations, our hypothesis was that QPI could detect and distinguish these processes. We validated this approach across various models, analyzing the dynamic behavior of cell populations exposed to different agents, including antivirals, nanomaterials, and cytotoxins.

Resistance to cell death is a hallmark of cancer. While autophagy's dualistic role in tumor biology is well known, our preliminary results suggest it mainly supports adaptation and resistance rather than inducing cell death. We hypothesized that autophagy, cell-in-cell formations, and polyploidization contribute to survival, remodeling, and dedifferentiation into a pluripotent state, often linked to tumor self-renewal and resistance. Our focus was on how prolonged stress (reactive oxygen species, starvation) affects autophagic flux and the involvement of autophagy in cancer cell dedifferentiation and self-renewal.

Given autophagy's key role in chemotherapy resistance, exploring autophagy inhibitors as adjunct therapies is promising. These inhibitors are also considered for other diseases, e.g., hydroxychloroquine for COVID-19. Since endosomal pathways, autophagy, lysosomal degradation, and extracellular vesicle secretion are interconnected, our research examined how various inducers and inhibitors influence these processes, particularly their effect on extracellular vesicle composition and soluble factors like cytokines and chemokines in head and neck cancer cells.

This habilitation thesis includes selected publications from 2014 to 2024, in which I contributed as first, corresponding, or co-author. All works revolve around cancer cell death, resistance mechanisms, and their connection to cellular secretion. The research primarily explores various forms of cell death in cancer, including resistance to oxidative stress and autophagy, with a focus on methods for identifying and differentiating these processes across different cancer cell lines.
The subsequent sections examine the relationship between autophagy, cell death pathways, repair mechanisms, and extracellular vesicle secretion, especially phosphatidylserine-positive extraellular vesicles involved in intercellular communication.

[1] The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

[1][2] Raudenská, M., Balvan, J. & Masařík, M. Cell death in head and neck cancer pathogenesis and treatment. *Cell Death and Disease* 12, 192 (2021).

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20 %	30 %	50 %	70 %

[2] Vicar, T., Balvan, J., Jaroš, J., Jug, F., Kolář, R., Masařík, M., Gumulec, J. Cell segmentation methods for label-free contrast microscopy: review and comprehensive comparison. *BMC bioinformatics* **20**, 360, (2019).

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
40 %	70 %	30 %	70 %

[3] Vicar T, Raudenska M, Gumulec J, Balvan J. The quantitative-phase dynamics of apoptosis and lytic cell death. *Scientific Reports*, 10, 1566 (2020)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70 %	80 %	50 %	90 %

[4] Balvan J, Krizova A, Gumulec J, Raudenska M, Sladek Z, Sedlackova M, Babula, P., Sztalmachová, M., Kizek, R., Chmelík, R., Masařík, M. Multimodal holographic microscopy: Distinction between apoptosis and oncosis. *PLoS ONE*

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
80 %	80%	40 %	90 %

[5] Fojtů, M., Balvan, J., Raudenská, M., Vičar, T., Bouša, D., Sofer, Z., Masařík, M., Pumera, M. Black Phosphorus Cytotoxicity Assessments Pitfalls: Advantages and Disadvantages of Metabolic and Morphological Assays. *Chemistry – A Europian Journal*, **25**, 349–360 (2019)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20 %	10 %	20 %	50 %

[2] Bibliographic record of a published scientific result, which is part of the habilitation thesis.

[6] Eyer, L.; Svoboda, P.; Balvan, J.; Vičar, T.; Raudenská, M.; Štefánik, M.; Haviernik, J.; Huvarová, I.; Straková, P.; Rudolf, I.; Hubálek, Z.; Seley-Radtke, K.; de Clercq, E.; Růžek, D. Broad-Spectrum Antiviral Activity of 3′-Deoxy-3′-Fluoroadenosine against Emerging Flaviviruses. *Antimicrobial Agents and Chemotherapy*, 65, e01522-20, (2021)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10 %	10 %	20 %	25 %

[7] Balvan, J., Gumulec, J., Raudenská, M., Křížová, A., Štěpka, P., Babula, P., Kizek, R., Adam, V., Masařík, M. Oxidative stress resistance in metastatic prostate cancer: Renewal by self-eating. *PLoS One* **10**, e0145016 (2015).

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
90 %	50 %	50 %	90 %

[8] Gumulec, J., Balvan, J., Sztalmachová, M., Raudenská, M., Dvořáková, V., Knopfová, L., Polanská, H., Hudcová, K., Ruttkay-Nedecky, B., Babula, P., Adam, V., Kizek, R., Stiborová, M., Masařík, M. Cisplatin-resistant prostate cancer model: Differences in antioxidant system, apoptosis and cell cycle. *International Journal of Oncology.* **44**, 923–933 (2014).

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
60 %	10 %	20 %	40 %

[9] Raudenska M, Balvan J, Masarik MJMC. Crosstalk between autophagy inhibitors and endosome-related secretory pathways: a challenge for autophagy-based treatment of solid cancers. *Molecular Cancer*, 20(1):1–27 (2021)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50 %	50 %	50 %	90 %

[10] Hanelova K, Raudenska M, Masarik M, Balvan J. Protein cargo in extracellular vesicles as the key mediator in the progression of cancer. *Cell Communication and Signalling*, 22 (2024)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10 %	70 %	50 %	90 %

[11] Hanelova, K., Raudenska, M., Kratochvilova, M., Navrátil, J., Vičar, T., Bugajová, M., Gumulec, J., Masařík, M., Balvan, J. Autophagy modulators influence the content of important signalling molecules in PS-positive extracellular vesicles. *Cell Communication and Signalling* **21**, 120 (2023)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50 %	90 %	50 %	90 %

[12] Bugajova, M., Raudenska, M., Hanelova, K., Navratil, J., Gumulec, J., Petrlak, F., Vicar, T., Hrachovinova, S., Masarik, M., Kalfert, D., Grega, M., Plzak, J., Betka, J. Balvan, J. Glutamine and serum starvation alters the ATP production, oxidative stress, and abundance of mitochondrial RNAs in extracellular vesicles produced by cancer cells. *Scientific Reports* **14**, 25815 (2024)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50 %	70 %	50 %	90 %