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# Translational Research in Oncology and the Chances for Interdisciplinary Cooperation

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# Contents

# Section A

## Abstracts to the conference presentations

# Translational research (urothelial carcinoma) and the chances for interdisciplinary cooperation

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***Keywords:*** *Urothelial carcinoma; neoadjuvant setting; cisplatin-based chemotherapy; response; resistance.*

The center of translational research consists in solving the problems generated by current medical practice, using methodologies and techniques of experimental sciences and vice versa, verification of experimental procedures in clinical conditions. The objective of this presentation is to define the major clinical problems we are facing in the field of urothelial carcinoma treatment and introduce the patients´ databases and archived samples of frozen plasma, urine, and tumors collected over the last decade, now awaiting our common research. Urothelial carcinoma, also known as transitional cell carcinoma (TCC), is by far the most common type of bladder cancer. These cancers start in the urothelial cells that line the inside of the bladder, but also other parts of the urinary tract, such as the part of the kidney that connects to the ureter (the renal pelvis), the ureters, and the urethra. Majority of patients have non-muscle-infiltrating carcinomas and are treated surgically (bladder tumor transurethral resection) with addition of local therapy (cytotoxic drugs or Bacillus Calmette–Guérin vaccine). Treatment of both, local and locally advanced muscle-infiltrating disease is based on radical cystectomy with prior neoadjuvant cisplatin chemotherapy with achievement of pathological complete remissions up to 50%. This means that a half of patients do not respond to chemotherapy optimally or at all and therefore, they are the subjects of our translational research interests. Another current clinical major issue is represented by patients with inoperable locally advanced or metastatic urothelial carcinoma who are still, despite the significant advances in this field, treated with first line cisplatin-based chemotherapy with objective response rates up to 60% since the end of the last century. The subjects of our translational research interests are the patients directly progressing on this chemotherapy because they are primarily refractory to the key drug cisplatin.

# Bioinorganic materials and their application perspectives in clinical medicine

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***Keywords:*** *Inorganic materials; 3D printing; bone substitute; inorganic nanoparticles; nanoparticle signal enhancement.*

The development of new bioinorganic materials is steadily attracting attention of the researchers as frontier research and create breakthroughs fields in several multidisciplinary sciences, including tissue engineering applications. Bioinorganic materials may find their application as bulk substance in tissue scaffolds for e.g. the hard bone replacements, or in the form of nanoparticles used for diagnostic purposes. The bulk applications are mostly based on hydroxyapatite (HAp) which represent the substance of natural bone material. However, for practical utilisation HAp requires its improvement with respect to bioactivity and mechanical properties. Up-to-date application of HAp include production of the scaffolds that are produced by 3D printing technologies. The on-site production of the required bone substitute seems to be promising and patient-friendly technology respecting individual specific biological shape individualities. The easy availability and production can significantly improve patient chances after accident and/or arthroplasty joint revision, war zone victims or tumour patient replacements. As nanoparticles after modification, have been successfully validated in the imaging of arteriosclerosis and cardiovascular diseases, but also in the imaging of tumours and their microenvironment using a stimulated response to inorganic nanomaterials. One of the significant applications being developed is surface-enhanced Raman scattering for imaging tissues or the cells. The nanoparticles excellent specific optical and/or photothermal response can be applied for localizing noble metal or doped oxide nanoparticles within the cell structure for different cell substance presence reporting.

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# Section B
## Full texts

# Predictive biomarkers of urothelial carcinoma and the possibilities of interdisciplinary cooperation

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***Keywords:*** *Urothelial carcinoma; prediction; prognosis; biomarkers; selection of patients.*

Bladder cancer is the ninth most common malignancy with a gross incidence of 20.4 cases per 10,000 inhabitants. The most common bladder cancer is urothelial carcinoma, which accounts for 90% of all malignant tumours. At the time of diagnosis, 30% of patients have a muscle-infiltrating carcinoma (MIBC) [1]. The gold standard for the treatment of local (T2) and locally advanced (T3-4N+) MIBC is radical surgery. The five-year survival of patients with MIBC is 50% despite adequate treatment. To improve treatment outcomes, combined neoadjuvant chemotherapy (NAC) based on cisplatin (CDDP) was introduced into clinical practice, leading to increase of 5-year overall survival (OS) by 5% and disease-free survival (DFS) by 9% [2]. For patients who are not candidates for radical surgery (comorbidities, poor performance status), concomitant chemoradiotherapy is an alternative local treatment after NAC. A major clinical problem remains that 50-60% of patients do not respond to neoadjuvant treatment [3]. Thus, this patient population is unnecessarily exposed to the toxic effects of chemotherapy and at the same time, definitive local treatment is delayed. Until now, there are no predictive biomarkers, on basis of which we would be able to pre-select these non-responders.

Metastatic urothelial carcinoma (MUC) is an incurable disease. Patients with good performance status (ECOG 0-1) treated with first line CDDP-based chemotherapy have OS of 13-15 months with objective response rate of 50-60% [4,5].

In CDDP-unfit patients (impaired renal function, functional hearing impairment, poor performance status - ECOG 2 or more, comorbidities), carboplatin-based chemotherapy with the objective response rate of 40% and a OS of 9 months is used [6]. Based on the phase 2 studies [7, 8], the use of check-point inhibitors – both monoclonal antibodies, pembrolizumab against

protein-1 programmed death (PD-1) and atezolizumab against PD-1 ligand (PD-L1) might also be considered.

The future of the standard first-line treatment of patients with MUC (both CDDP-fit and CDDP-unfit) will probably be a combination of chemotherapy with immunotherapy [9] and a combination of immunotherapy with an agent to target nectin-4 [10].

A major clinical problem represents patients who do not achieve an objective response to systemic therapy or do progress directly on treatment. To this day, there are no predictive biomarkers, on basis of which we would be able to pre-select these non-responders.

Among predictive/prognostic markers of urothelial carcinoma studied at National Cancer Institute (Bratislava, Slovakia) in past decade belong plasma antioxidants and thiobarbituric acid reactive substances [11,12], systemic immune-inflammation index [13-15], endogenous DNA damage in peripheral blood mononuclear cells [16,17], and venous thromboembolism [18].

**Acknowledgements**

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**References**

[1] Stenzl A, Witjes JA, Compérat E, *et al.* Guidelines on Bladder Cancer Muscle-invasive and Metastatic. 2012. Available at: https://uroweb.org/wp-content/uploads/07_Bladder-Cancer_LR-Feb-17th.pdf (24-08-2020).

[2] Advanced Bladder Cancer (ABC) Meta-analysis. Collaboration.Adjuvant chemotherapy in invasive bladder cancer: A systematic review and meta-analysis of individual patient data. Eur Urol 2005; 48(2): 189-201.

[3] Zargar H, Shah JB, van Rhijn BW, *et al.* Neoadjuvant dose dense mvac versus gemcitabine and cisplatin in patients with cT3-4aN0M0 bladder cancer treated with radical cystectomy. J Urol 2018;199(6): 1452-1458. doi: 10.1016/j.juro.2017.12.062

[4] Loehrer PJ, Einhorn LH, Elson PJ, *et al.* A randomized comparison of cisplatin alone or in combination with methotrexate, vinblastine, and doxorubicin in patients with metastatic urothelial carcinoma: cooperative group study. J Clin Oncol 1992; 10(7): 1066-1073.

[5] von der Maase H, Hansen SW, Roberts JT Dogliotti L, *et al.* Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: re-sults of a large, randomized, multinational, multicenter, phase III study. J Clin Oncol 2000; 18(17): 3068-3077.

[6] De Santis M, Bellmunt J, Mead G, *et al.* Randomized phase II/III trial assessing gemcitabine/carboplatin and methotrexate/carboplatin/vinblastine in patients with advanced urothelial cancer who are unfit for cisplatin-based chemotherapy: EORTC study 30986. J Clin Oncol 2012; 30(2): 191-199.

[7] Balar AV, Castellano D, O'Donnell PH, *et al.* First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. Lancet Oncol 2017; 18(11): 1483-1492.

[8] Balar AV, Galsky MD, Rosenberg JE, *et al.* Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet 2017; 389(10064): 67-76.

[9] Grande E, Galsky M, Arranz Arija JA, *et al.* IMvigor130: efficacy and safety from a phase 3 study of atezolizumab (atezo) as monotherapy or combined with platinum-based chemotherapy (PBC) vs placebo + PBC in previously untreated locally advanced or metastatic urothelial carcinoma (mUC). Ann Oncol 2019; 30(5): v888.

[10] Hoimes CJ, Rosenberg JE, Srinivas S, et al. EV-103: Initial results of enfortumab vedotin plus pembrolizumab for locally advanced or metastatic urothelial carcinoma. Ann Oncol 2019; 30(5): mdz249.

[11] Palacka P, Kucharska J, Mego M, *et al.* Antioxidant stimulation as the adaptation mechanism in patients with metastatic urothelial cancers (MUC) treated with gemcitabine and cisplatin (GC). J Clin Oncol 2013;31(6): 281.

[12] Slopovsky J, Kucharska J, Obertova J, *et al.* Marker of lipid peroxidation TBARS predicts survival in patients with metastatic urothelial carcinoma (MUC). J Clin Oncol 2020; 38(15): e17023.

[13] Palacka P, Mego M, Mikulova T, *et al.* Systemic immune-inflammation index predicts survival in Caucasian patients with metastatic urothelial carcinoma. J Clin Oncol 2017; 35(15): e16015.

[14] Palacka P, Katolicka J, Albertova T, *et al.* Systemic immune-inflammation index to predict survival in patients with metastatic urothelial carcinoma treated with second line vinflunine. J Clin Oncol 2020; 38(15): e17019.

[15] Obertova J, Palacka P, Gallik D, *et al.* Systemic immune-inflammation index to predict survival in muscle-infiltrating urothelial carcinoma. J Clin Oncol 2020; 38(15): e17022.

[16] Palacka P, Sestakova Z, Kalavska K, *et al.* Endogenous DNA damage in peripheral blood lymphocytes (PBLs) in patients with metastatic urothelial carcinoma (MUC). J Clin Oncol 2018; 36(15): e16511.

[17] Palacka P, Sestakova Z, Holickova A, *et al.* Endogenous DNA damage levels in peripheral blood mononuclear cells in patients with muscle-infiltrating urothelial bladder carcinoma. J Clin Oncol 2019;37(15): e16020.

[18] Palacka P, Luha J, Mego M, *et al.* Venous thromboembolism in metastatic urothelial carcinoma. J Clin Oncol 2016; 34 (15); e16029.

# ZnO nanoparticles in modern oncology

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***Keywords:*** *ZnO nanoparticles; oncology, tumour suppressing.*

Novel diagnostic and therapeutic approaches in modern oncology using nanoparticles of semi-conducting oxides allow enhancing contrast for lymph nodes and bone marrow, perform anti-microbial and antifungal activity but can be used for targeting the drug and gene delivery. Zinc (Zn) as an essential trace element of the human body as well as a co-factor of more than 300 mammalian enzymes is a powerful tool for controlling biochemical reactions. Zinc has an tumour suppressive effect in malignant prostate and breast cancer cells. Hepatocellular carcinoma has a downregulated functional Zn uptake transporter (ZIP-14) and the Zn line has been shown to inhibit cell proliferation. For the pancreatic adenocarcinoma, downregulated ZIP-3 located in ductal and acinar epithelium is responsible for proliferation and the zinc treatment as a strong tool can be applied. On the other hand, pure or salt forms of Zn source does not need to be resorbed or accumulated by tissue. For this reason, it is suitable to combine Zn with a secondary transporter, or to introduce it in the form of a sparingly soluble substance such as oxide with a high affinity for proliferating cells. ZnO nanoparticles belong to the group of biocompatible materials with a relatively simple method of solvothermal/hydrothermal preparation, but extensive possibilities of physical induction of cytotoxicity. ZnO nanoparticles may have positively charged surface in the physiological environment. In contrast, cancer cells incorporate large amounts of negatively charged molecules, receptors, and proteins into their outer membranes. Due to this property, selective cytotoxicity can be induced. Phospholipid bilayer coated ZnO nanoparticles can be used in photodynamic therapy. In the case of radiation medicine, ZnO is a significant challenge for use as a radio sensitizer after doping. This work discusses the most current possibilities of ZnO from the point of view of nanomedicine, toxicity pathways of ZnO but also future challenges of the use in cancer therapy.

## Introduction

Nanoparticle systems are essential probes in modern medicine, including oncology. Nanoparticle preparation processes have undergone extensive development in recent decades, from the preparation of pure and homogeneous nanoparticle systems with the desired dimensional distribution to the preparation of heterogeneous mixtures of nanoparticle systems coated with different functionalised coatings. There is an evidence of a low intracellular Zinc (Zn) concentration in various cancers, such as prostate cancer, hepatocellular carcinoma, pancreatic adenocarcinoma, ovarian carcinoma, and others. [1] Zn has been overlooked in connection with cancer, and therefore, there are only a few sources describing physiology and biochemistry in mammals. However, the fact is that research in this area has so far been carried out only sporadically, because of the higher focus of scientific capacities by studies and the optimization of conventionally used anticancer agents and chemotherapies. Due to the low number of scientific works dealing with the basic role of Zn lines in tumorigenesis, it is also not easy to obtain funding [1]. It must be emphasized that this work does not define Zn and $Zn^{2+}$ or nanoparticles of ZnO as a unique cancer therapy, but we would like to exclusively discusses the currently available knowledge on the relationship of Zn and ZnO nanoparticles to proliferation. As an example, we have chosen advanced prostate cancer, pancreatic adenocarcinoma, and hepatocellular carcinoma.

## Zn in cancer therapy and diagnostics

Number of studies have shown an approximately 60 % - 80 % reduction of intracellular $Zn^{2+}$ level in prostate malignant tissue compared to benign tissue (0.2 mM - 0.8 mM). In contrast to this amount, there is only an exceptional report that neoplastically transformed tissue contains a comparable level of $Zn^{2+}$ as healthy tissue. The reduction in Zn is even demonstrated in premalignant cells and the tissues surrounding malignant cells [2, 3]. Based on low level observations, several cytotoxicity and tumour suppressive studies of Zn have been performed on prostate cells. The effects are categorized according to the work of Costello and Franklin [4-6]:

1. Inhibition of growth and proliferation (presence of zinc during apoptosis and cell death).

2. Altered metabolic effects (inhibition of citrate oxidation, essential for synthetic processes and tumour energy).

3. Inhibition of invasion and migration (according to the 1. and 2.).

The second important discussion point is the question of whether the reduction in the amount of $Zn^{2+}$ occurs due to the presence of cancer or whether it is a consequence of the proliferation. Decreased $Zn^{2+}$ levels in prostate cancer is one of the very early events in premalignant cells, indicates that these changes precede malignancy, and do not result from malignancy [1]. To support this claim, reducing the amount of $Zn^{2+}$ does not lead to malignancy in the absence of oncogenic transformation of the normal cell. It is hypothesized that decreased expression of the functional zinc uptake transporter (ZIP1) is associated with decreased of $Zn^{2+}$ level during proliferation and therefore is a suitable target for further, more representative studies [1-3]. In the case of hepatoma cells in hepatocellular cancer, a 60 % decrease in $Zn^{2+}$ level and downregulation of ZIP14 was demonstrated by several works compared to healthy cells [1-3]. Downregulation of transported was observed in early-stage well-differentiated malignancies and persist in the advanced stage malignancies.

In contrast to the limited and contradictory pieces of information describing ZIP1 in prostate tissue, the tumour suppressor effect of $Zn^{2+}$ together with inhibition of proliferation has been consistently described for a wide scale of cancer types [7, 8]. However, for other selected types of cancer, the relationships of ZIP expression to proliferation are not so controversial. In the case of pancreatic adenocarcinoma, reduced activity of the ZIP3 transporter was demonstrated as well as reduced activity of ZIP14 in hepatocellular carcinoma. In these cases, $Zn^{2+}$ treatment leads to a representative inhibition of tumorigenesis. Even if functional uptake transporter differs, both hepatocellular and pancreatic cancers reveal transporter expression decrease and thus $Zn^{2+}$ is related to proliferation and therefore the question arises how effective treatment can be with a targeted increase in the intracellular concentration of $Zn^{2+}$. To support this contention, Lightman *et al.* reported the similar finding for ovarian tumours when compared with benign tissue. In contrast, for breast cancer, a significantly higher level of $Zn^{2+}$ was observed in the malignant tissue compared to benign [9-12]. At elevated $Zn^{2+}$ level, overexpression of the ZnT2 transporter occurs, which causes compartmentalization of accumulated zinc, leading to protection of tumour cells against zinc cytotoxicity. This effect has not been demonstrated in prostate,

liver, and pancreatic cancer. According to that finding, one can imply that preventing the zinc compartmentalization together with higher intracellular zinc level may significantly enhance cancer therapy. We also call for further study dealing with the suppression of the pathways responsible for compartmentalization, together with high intracellular zinc level treatment.

However, the way in which zinc can reach the targeted tissue remains a problem. Although exact mechanism for inducing apoptosis is not clear, mutation or damage to DNA appears to play a major role in triggering activation of the p53 gene (tumour suppressing factor), which leads to apoptosis [13]. The specific DNA-binding domain of p53 contains a complex tertiary structure that is stabilized by $Zn^{2+}$. Thus, zinc plays a major role in maintaining the activity of tumour suppressor gene p53, which regulates apoptosis of the cells [13]. Similarly, zinc also plays a crucial role in the activation of the caspase-6 enzyme, a major enzyme responsible for apoptosis. Caspase-6 is the most sensitive apoptosis-related molecular target of $Zn^{2+}$ [14]. It is responsible for the activation of caspase-3 and other enzymes that are responsible for nuclear membrane dissolution leading to cell death [15]. While low concentrations of $Zn^{2+}$ can cause proliferation, high concentrations of $Zn^{2+}$ have been shown to cause apoptosis regardless of whether the tissue is malignant or benign. Finally, from an expert view, we can consider $Zn^{2+}$ level as an important event in tumorigenesis. Let's take a look on opportunities of zinc in a form of ZnO.

**ZnO nanoparticles in cancer therapy and diagnostics**

The main feature of zinc oxide (ZnO) is the band structure, resulting from the different arrangement and overlapping of energy bands. However, this does not mean that all the properties of pure metal have disappeared. ZnO nanoparticles represent a nano-sized crystalline form of ZnO, with a size up to 200 nm. Known methods of preparation include e.g. hydrothermal/solvothermal syntheses, pyrolysis micro-emulsion methods and others. Nanoparticles of ZnO are characterized by a large surface to volume ratio and thus the ability to aggregate. To prevent aggregation and keep individual nanoobjects in dispersion, it is necessary to stabilize nanoparticles in solution using suitable surface-active agents. Pure form of ZnO and its nanoparticles have high biocompatibility level and are generally recognized as safe by the FDA [16]. The administered ZnO (dosage does not exceed lethal level) can be easily biodegraded by lysosomes or can be utilised in the active nutritional cycle of the body [17]. Pure $Zn^{2+}$ has been

shown to be cytotoxic for selected tumour tissues, but the method for its selective internalization into targeted tissue has not been properly clarified by ani scientific work group. Unlike pure zinc, ZnO nanoparticles have selective toxicity to cancer transformed cells but also selective affinity for proliferating tissue [18]. According to the Bish and Rayamajhi, extracellular ZnO shows biocompatibility, but elevated levels of administered intracellular ZnO show enhanced cytotoxicity through zinc-mediated protein activity disequilibrium and oxidative stress [16]. Due to the semiconducting nature of ZnO, induction of the oxidative stress and damage inside the cell is possible. ZnO generates reactive oxygen species (ROS) and induces cell death when the anti-oxidative capacity of the cell exceeded [19]. It was reported that $Zn^{2+}$ released from ZnO NPs inhibits enzymes in the TCA cycle resulting in a reduction in citrate concentration a leading to the same apoptosis mechanism described for pure $Zn^{2+}$.

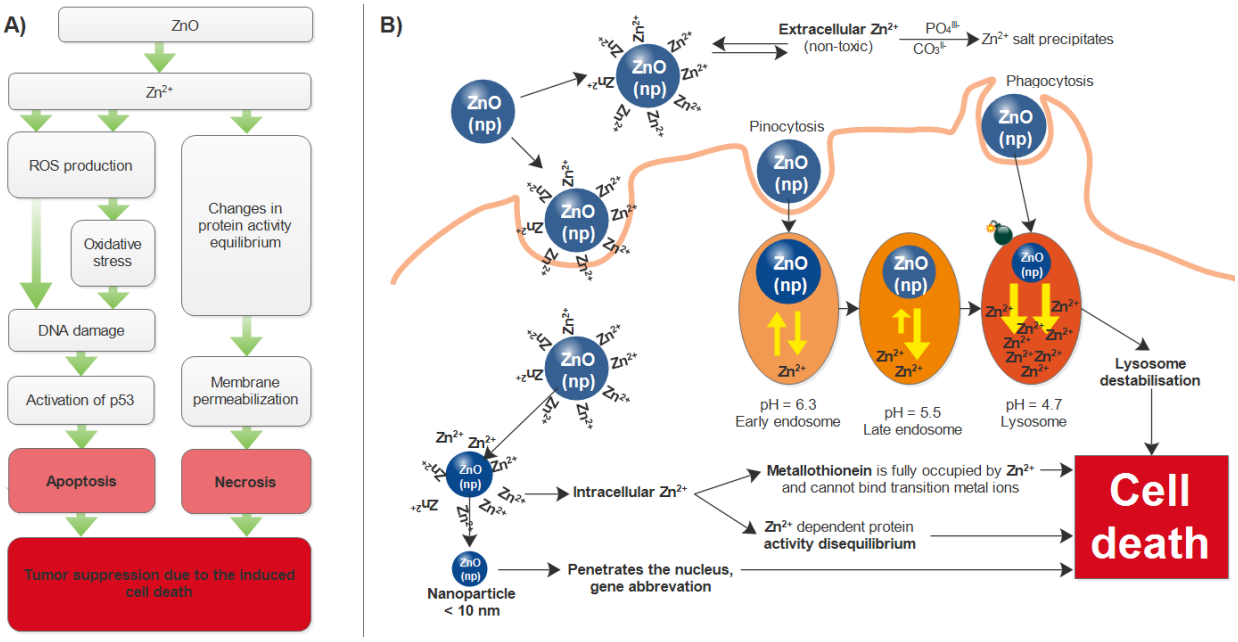
Ideal nanoparticles size for the tumour suppression has been found within the interval 10 nm -100 nm. This interval is based on the fact that objects smaller than 10 nm are immediately excreted by the kidneys due to the kidney´s averaged sieving coefficient. Other studies have shown that nanoparticles up to 100 nm show greater efficacy in inhibiting proliferation [16]. Decreasing size of the nanoparticles leads to a deeper penetration of the tumour tissue, but the fact remains that the toxicity to healthy cells at dimensions below 10 nm increases significantly by means of increased diffusion through the cytoplasm [20].

Tissue resident macrophage in the liver and spleen rapidly clears most of nanoparticles entering the blood by recognising the natural proteins called “opsonins” adsorbed on the particles immediately after entering the bloodstream [20]. These ultra-sized particles under 5 nm even enter the nucleus and show toxicity through the nucleus aberration, that is absolutely unfavourable for health cells. Unlike pure $Zn^{2+}$ easily enter the cell by diffusion, nanoparticles interact at the nano-level with the cell surface. Nano-bio interface is a dynamic physicochemical interaction between the nanomaterial surface and the surface of biological components, which deals with the kinetic and thermodynamic exchanges [21]. It includes the interaction between the nanoparticles’ surfaces, the solid-liquid interface, and the solid liquid interface contact zone with the biological membrane.

Nanoparticles without suitably modified surfaces that have entered the body immediately form a kind of corona from surrounding proteins. This corona is called protein-corona. Compo-

sition of the corona depends on nanoparticle size, hydrophobicity, surface charge of the nanoparticle but also on surrounding proteins and peptides. This is a most simple kind of natural coating, which allows the immune system to recognize nanoparticle, prevent or induce aggregation in the bloodstream [22]. There is a dynamic interaction between corona-proteins bound to the ZnO nanoparticle and the environment. Studies of corona-proteins in different tissues make it possible to adjust the properties and coating of ZnO nanoparticles for the most specific targeting.

**Figure 1.** (A) Schematic of the procedure where ZnO induces cell death from a chemical point of view and (B) scheme of macroscopic ZnO nanoparticles cellular release.




The mechanism of cytotoxicity of ZnO is the release of $Zn^{2+}$ cations after endocytosis of the nanoparticle into the cell (Fig. 1). Soluble extracellular zinc shows no or very little cytotoxicity and almost no possibility of selective targeting. Recent research shows that extracellular $Zn^{2+}$, when exposed to cell culture and media, forms poorly soluble amorphous zinc-carbonate phosphate precipitates as a cell protection against $Zn^{2+}$ overload (Fig 1.) [23]. On the contrary, the release of $Zn^{2+}$ inside the cell does not lead to precipitation but to the initiation of regulatory cascades (**Figure 1.**) [23].

## Coating of ZnO nanoparticles

Modern science also allows the packaging of individual nanoparticles in inorganic or organic coatings. This coating may contain various molecular tags enabling targeted and highly selective resorption by a targeted tissue. There are three basic types of nanoparticle coating, protective, functionalizing or stabilizing [24]. Currently used coatings are briefly summarized in the table (**Table 1**.) [24].

The protective coating provides protection of the nanoparticle against the external environment or, conversely, protection of the external environment against the adverse effects of the nanoparticles. Nanoparticle systems are often prepared as dispersions in liquid media, their stabilization against sedimentation or spontaneous aggregation is important. In the case of pure metal nanoparticles, it is appropriate to use citric acid. For oxide nanoparticles such as ZnO, it is appropriate to use polymer coatings. The functionalized coating can be used to adjust the hydrophilicity of the surface, by the interaction of the functional groups of the coating with the solvent.

## Coating of ZnO nanoparticles for medical applications

The most complex coatings are prepared for medical purposes and include a combination of a protective and functionalizing layer. This kind of coating can provide biocompatibility, controlled time of presence in the blood, can simplify separation of particles from blood or transport particles selectively to the targeted organ. The targeting of nanoparticles to specific organs and tissues is extremely important. A phospholipid bilayer can be used to prevent the dissolution of the nanoparticle during transport by blood containing specific antibodies. This model simulates a liposome transporting lightweight molecules and is subsequently resorbed by a target tissue recognizing the attached antibody [25]. The presence of multicoating, a functionalized PEG envelope equipped with antibodies, allows the nanoparticle to persist longer in the blood vessels. Another possible coating is based on dextran and allows the specific absorption of the particles by the liver.

**Table 1.** Current possibilities of ZnO nanoparticle coating

| Reason for coating | Coating material |
|---|---|
| Increase in stability | Polymers, Surfactants |
| Enhanced wettability | Polymers, Inorganic layers |
| Prevention against dissolution | Mostly $SiO_2$ |
| Improvement of physical and chemical functions | $SiO_2$ or inorganic layers. Increasing of fluorescence by saturating free surface bonds (mainly for QDs) |
| Cost savings | Ag, Pt, Pd, Rh. Application of thin incomplete layer of expensive catalyst on |
| Protecting | Organic layers. Protection against catalytic poisons |
| Biocompatibility and functional-ization | Antibodies, Inorganic layers, biocompatible polymers, peptides, saccharides |

**Coating of ZnO ultra-sized nanoparticles for a tumour visualization**

Quantum dots (QDs) are ultra-sized nanoparticles with a unique fluorescence property whose wavelength depends on the size of the nanoparticle. Due to the width of their band gap (Eg = 3.4 eV; UV region of electromagnetic radiation), QDs of ZnO emit light in the visible electromagnetic regime. The quantum yield of fluorescence is even an order higher than the quantum yield of an equivalent amount of the fluorescein isocyanate (FITC; contrast agent commonly used today). QDs of ZnO are significantly less sensitive to fluorescence quenching than FITC or another organic fluorescent marks. Significant advantage is also a sparing solubility of ZnO QDs. Kim et al. described the use of phospine-coated QDs to map lymph nodes in

mice [26]. According to the work of Akerman et al. (YYYY), we can imply for ZnO QDs connecting of the nanoparticles to binding domain for endothelial cells in lung blood vessels (peptide chain is CGFECVRQCPERC) will result in endocytosis of the packaged ZnO QDs. Thus, internalized QDs can be used to visualize tumours and metastatic foci located inside the lung [27]. Gao et al. formed a coating for QDs, of an amphiphilic copolymer that was stabilized against aggregation by tri-n-octylphosphine oxide [28]. A copolymer is attracted to the hydrophobic surface of tri-n-octylphosphine oxide in solution. The copolymer was a triblock, consist of butylacrylate, ethylacrylate, and methacrylic acid segments [28]. The first two segment types are more hydrophobic than the third, leading to the different solvation [28]. 30 nm long PEG chains were attached to the hydrophilic chain of the copolymer [28]. Tumour targeting was provided by an antibody against prostate specific membrane antigen. QDs were then injected into the bloodstream. In this study, zero accumulation was observed in the lungs, kidneys, and brain, but lung blood vessels were clearly observable.

## Conclusion

In the light of current knowledge and literature sources, it is evident that intracellular zinc levels significantly affect tumorigenesis and act as a suitable marker for the detection of preproliferating tissue. Appropriately prepared ZnO nanoparticles should be prospectively a suitable choice for targeted visualization, but also for targeted drug delivery and thus treatment of selected cancer types.

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## References

[1] L. C. Costello, R. B. Franklin, *Expert Review of Anticancer Therapy,* **2012** ,12, 1.

[2] R. B. Franklin, P. Feng, B. C. Milon and et al., *Mol Cancer.,***2005** 4, 32.

[3] M. Cortesi, E. Fridman, A. Volkov, *Prostate,* **2008,** 68, 994–1006.

[4] L. Costello and R. B. Franklin, *Mol Cancer.,* **2006.** 5, 17.

[5] R. B. Franklin, B. Milon, P. Feng , L. C. Costello, *Front Biosci,***2005,** 10, 2230–2239.

[6] L. C. Costello, R. b. Franklin, P. Feng, M. Tan, O. Bagasra **2005,** 16, 901–915.

[7] J. Lemire, R. Mailloux ,V. D. Appanna, *J Appl Toxicol,* **2008,** 28, 175–182.

[8] J. Xu, Y. Xu, Q. Nguyen, P. M. Novikoff , M. J. Czaja, *Am J Physiol,* **1996,** 270, G60-G70.

[9] S. L. Rizk, H. H. Sky-Peck, *Cancer Res,* **1984,** 44, 5390–5394.

[10] P. M. Santoliquido, H. W. Southwick, J. H. Olwin, *Gynecol Obstet,* **1976**,142, 65–70.

[11] E. J. Margalioth, J. G. Schenker, M. Chevion, **1983,** 52, 868–872.

[12] M. J. Farquharson, A. Al-Ebraheem, K. Geraki, R. Leek, A. Jubb, A. Harris, *Phys Med Biol,* **2009,** 4213–4223, 54.

[13] K. W. Ng, S. P. Khoo, B. C. Heng, M. I. Setyawati, E. C. Tan, X. Zhao ,*Biomaterials,* **2011***,* 32, 32, 8218-8225.

[14] D. K. Dhawan ,V. D. Chadha, *Indian J Med Res,* **2010,** 132, 676-82.

[15] D. Beyersmann, *Materialwissenschaft und Werkstofftechnik,* **2002,** 33, 12.

[16] G. Bisht and S. Rayamajhi, *Nanobiomedicine,* 2016.

[17] J. Zhou, N. S. Xu, Z. L. Wang, *Advanced Materials,* **2006**, 18, 18, 2432.

[18] C. Hanley, J. Layne, A. Punnoose, K. M. Reddy, I. Coombs, A. Coombs, *Nanotechnology,* **2009,** 19, 29, 295103.

[19] J. W. Rasmussen, E. Martinez, P. Louka , D. G. Wingett, *Expert Opin Drug Deliv,* **2010,** 7, 9, 1063-77.

[20] K. Huang, H. Ma, J. Liu, S. Huo, A. Kumar, T. Wei, *ACS nano,* **2012,** 6, 5, 4483-93.

[21] A. E. Nel, l. Madler, D. Velegol, T. Xia, E. M. Hoek, P. Somasundaran, *Nat mater,* **2009** ,87, 543-57.

[22] P. Verderio, S. Avvakumova, G. Alessio, M. Bellini, M. Colombo, E. Galbiati, *Nano-Bio-Interface Perspective,* **2014**, 3, 7, 957-76.

[23] T. W. Turney, M. B. Duriska, V. Jayaratne, A. Elbaz, S. J. O'Keefe, A. S. Hastings, *Chem Res Toxicol,* **2012,** 25, 10, 2057-66.

[24] N.A., DECHEMA e.V., [Online]. Available: https://nanopartikel.info/en/nanoinfo/cross-cutting/993-coatings-cross-cutting-section#literatur. [Accessed 8 **2020**].

[25] R. H. Muller, K. Mader, S. Gohla, *Eur J Pharm,* **2000,** 50, 161–77.

[26] S. Kim, Y. T. Lim, E. G. Soltesz, *Nat Biotechnol,* **2004,** 22, 93–97.

[27] M. E. Akerman, W. C. W. Chan, P. Laakkonen, *Proc Natl Acad Sci,* **2002,** 99, 12617–21,

[28] X. H. Gao, Y. Y. Cui, R. m. Levenson, *Nat Biotechnol,* **2004,** 22, 969–76.

# Q-vent, a new low-cost device for emergency lung ventilation made by 3D printing of advanced nanomaterials

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The novel emergency lung ventilator (eALV) was designed during the COVID-19 crisis as a response of the low global availability of standard artificial lung ventilators (ALV) and aims to provide an open source basis for the rapid and emergency production of eALV. The device was developed in March 2020 as a volume-controlled ventilation, based on single-chip microcomputer. The breath mixture is expelled mechanically from the Ambu Breathing Bag® to a two-way valve separating the inhalation and exhalation. A standard endotracheal cannula is connected directly to the two-way valve. The regulation of maximum pressure or spontaneous inspiration and lung resistance is ensured by feedback pressure measurement in the form of monitoring the current load of the motor. The interconnection of the volume control and pressure monitoring prevents against barotrauma and volume trauma. It is possible to regulate tidal volume, number of breaths, inspiration to expiration ratio and end respiratory pause. The device is also equipped with an acoustic alarm to warn of spontaneous breathing, exceeding the allowable pressures or switching to a backup power source. The Q-vent device contains an integrated module for the mixture heater and humidifier. These modules, together with valves have been printed using 3D-print of a new antimicrobial materials developed for this purpose. The antimicrobial materials are based on a polymer matrix. Polymer matrix are different for each material and include polylactic acid (PLA) or polyethylene terephthalate-glycol (PET-G). Synthetic layered silicate Saponite (Kunimine Japan) with intercalated antimicrobial component Phloxine B was used as an antimicrobial component of the material. Described material prevents the formation or limits the formation of non-specific biofilms on the inner airways surface, that can be possible contaminated by breathing mixture. Also, a 3D-printed ceramic inserts with internal

(infilled) channels, heated to a high temperature, is used to eliminate pathogens from exhalation. In addition to the use of Q-vent during the coronavirus crisis, the device can be used in hard-to-reach terrains and in third world countries for lighter procedures such as caesareans and appendicitis.

## Introduction

Recent approaches in technological processes allows the preparation of complex, hybrid materials made of different components having different surface functionalization. Materials used in medicine includes mainly biocompatible materials or intelligent materials [1]. Functionalised materials are materials with adjusted physicochemical parameters. Otherwise, intelligent materials can respond to the stimuli from the surrounding environment (pressure, magnetic or electric field, etc.) [2]. So prepared materials have a possibility of use in a physiological or somatic environment as a replacement or protective elements. The possibility of adjusting physico-chemical properties of materials, to be suitable for use in physiological conditions is specific for biocompatible materials intended in manufacturing of artificial bone replacements, scaffolding for bone growth after osteosarcoma removal, creation of replacements for a skin tissue and more [3,4,5].

## Methods

One of the most commonly used method for the shaping of raw “home-made” or laboratory made materials into the final shape is rapid prototyping, including three-dimensional print (3D print). According to needs of the final application, 3D-printing uses several different methods including UV-resin hardening (SLA, SLS), powder sintering or classic thermoplastic filament printing (FDM, FFF) [6]. All methods are based on the similar, gradual application of material layers in precise geometric shapes (slices) and its subsequent solidification at the target site. The differences are in the solidification process (laser, solidification by cooling or drying, UV-curing) and the type of material (powder, filament or hydrogel, liquid). Different approaches allow use materials such as plastic, metal, ceramics, silicate composites with antimicrobial activity as well as encapsulated or dispersed living cells. In summary, three-dimensional print allows to produce 3D objects that are too expensive or impossible to manufacture by conventional methods [7].

There are two main methods of manufacturing thermoplastic filament from composite organic/inorganic materials. The first consists of melting the individually prepared components of

composite using double-screw extruder and subsequent extrusion of the thermoplastic printing filament. In this procedure, it is necessary to select the appropriate thermoplastic along with appropriate thermal and speed profiles of 3D-printer in order to avoid degradation of thermosensitive components during the printing [8,9].

The most commonly used thermoplastic for manufacturing the filaments are polylactic acid (PLA), acrylonitrile butadiene styrene (ABS), nylon, pure polyethylene terephthalate (PET) or its copolymer with glycol (PET-G) and thermoplastic polyurethane (TPU) [10,11]. These materials are commercially available mostly as powders or pellets into which other components can be easily mixed and subsequently formed into a composite printing filament. The advantage of 3D printing is the small amount of waste produced during production and the possibility of material production by recycling these plastics [12-14].

For a combination of different polymer matrices contains of different additives/hybrid compounds, it is possible to use two or more printing heads. Such an assembly allows to produce spatial structure with a non-homogeneous layout without the need for additional grouting which may devastate the product [15]. The accuracy of filament printing method can be up to 0.01 mm in the horizontal axes and more than a hundred times greater in the vertical axis.

For example, latest works in the field of medicine dealing with advanced 3D printing of a composite material based on layered silicate with encapsulated pancreatic cells that ensures insulin production and resistance to the immune tissue rejection. From the end of the 2019, there is also an evidence for the printing of functional hearth into the hydrogel described by Israel scientists. Another work is facing a challenge to produce thermoplastic photoactive nanomaterials as a composite with an aluminosilicate or hydroxyapatite functionalised by antimicrobial dye Phloxine B (PhB) [16]. So, prepared material reveals self-cleaning properties against pathogens after photo-sensibilization under the sunlight-irradiation. Dealing with this challenge was aim of this study. PhB is a derivate of fluorescein, containing xanthene and a chlorinated carboxyphenyl ring. Antimicrobial effect of PhB is based on induction of oxidative damage by forming the free radicals and reactive oxygen species (ROS) after irradiation with a wavelength of 537 nm (middle VIS spectrum) [17,18]. Toxic dosage of PhB is over 1.25 mg / kg of body weight [19].

PhB is usually used as an additive for colouring drugs or cosmetics in the USA and for a food colouring in Japan [20-22]. Recent scientific experiments clearly showed that PhB is great for visualisation of the cytoplasm and connective tissue. Antibacterial effect on gram-positive bacteria (G +), especially on methicillin-resistant Staphylococcus aureus, but also on bacteria such as Bacillus subtilis was described [23]. Growth reduction of Bacillus cereus occurs over the PhB concentration at level 25 μmol/l. Doubling the amount of PhB completely prevent the growth. The negative effect of PhB towards fungal growth has also been demonstrated [24].

The mechanism of the antimicrobial effect of PhB salt in an aqueous medium is the formation of an PhB anion, which binds to the positively charged components of bacterial membranes consisting of a peptidoglycan layer [25]. Light irradiation induces debromination. Hand in hand with debromination, large amount of singlet oxygen is formed, causing immediate oxidative stress and damage to the membrane components. Partial resistance of gram-negative (G-) bacteria exist due to the presence of lipopolysaccharides in their membranes. For G- bacteria, it was shown, that presence of ethylenediaminetetraacetic acid (EDTA) increases the permeability of membrane, leading to the similar adhesion of PhB, oxidative stress and subsequent bacteria death [26]. We have prepared one antimicrobial material based on PLA and second material based on PET-G as a thermoplastic matrix. Second additional component to the thermoplastic matrix was hybrid functionalised saponite (Sap). PhB was intercalated into the organically functionalised synthetic Sap matrix using cation exchange method. Described hybrid nanomaterial has up to 10 % higher antimicrobial activity than pure PhB, due to the natural antimicrobial activity of saponite. So prepared functionalised hybrid saponite particles with intercalated PhB are purple coloured. Hybrid saponite matrix was used to decrease hydrophobicity of thermoplastic matrix in the water environment.

## Conclusion

Hybrid material based on saponite was melt-compounded with PLA or PET-G pellets using double-screw extruder. Thermoplastic filament with 1.75 mm diameter was then extruded using single-screw extruder. This material was tested in contaminated environment and shown significant antimicrobial activity. It was not suitable to use pure thermoplastics with PhB, because arrangement of the polymer fibres inside the filament is so tight that prevents the interaction of PhB with water molecules on the surface [27]. Conversely, when saponite is used as a composite matrix for PhB, the surface availability of water is improved, leading to the enhanced overall

antimicrobial effect. The intercalation of PhB into the Sap ensures that there is no spontaneous release of PhB from the thermoplastic material structure. So prepared antimicrobial material was used to print components of the emergency lung ventilator Q-VENT, heart valve prototypes and cannulas.

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**References**

[1] X. Yang, Y. Hao and L. Cao, High Internal Phase Emulsions, 2020, 12, 4, 931.

[2] R. Wool, X. S. Sun, Bio-Based Polymers and Composites, 2005, 523-548.

[3] A. D. Drozdov, A. L. Kalamkarov, *International Journal of Solids and Structures*, 1996, 33, 29, 4411- 4429.

[4] K. Szurkowska, A. Laskus, J. Kolmas, Hydroxyapatite-Based Materials for Potential Use in Bone Tissu Infections, London: IntechOpen, Ltd., 2017.

[5] E. Salernitano, C. Migliaresi, *Journal of applied biomaterials & biomechanics*, 2003, 1, 3-18.

[6] W. C. Yan, P. Davoodi, S. Vijayavenkataraman, Y. Tian, W. C. Ng, J. Y. H. Fuh, K. S. Robinson, C.-H. Wang, *Adv Drug Deliv Rev*, 2018, 132, 270-295.

[7] Y. Wen, S. Xun, M. Haoye, S. Baichuan, C. Peng, L. Xuejian, Z. Kaihong, Y. Xuan, P. Jiang and L. Shibi, Biomater Sci, 2017, 22, 5(9), 1690-1698.

[8] V. Tuncay and P. M. A. v. Ooijen, "3D Printing for Heart Valve Disease: A Systematic Review," Eur Radiol Exp, vol. 15, no. 3(1), p. 9, 2019.

[9] M. Vukicevic, D. S. Puperi, K. J. Grande-Allen, S. H. Little, *Ann Biomed Eng*, 2017, 2, 508-519, 45.

[10] A. Lee, A. R. Hudson, D. J. Shiwarski, J. W. Tashman, T. J. Hinton, S. Yerneni, J. M. Bliley, P. G. Campbell,A. W. Feinberg, *Science*, 2019, 365,6452, ,482-487.

[11] G. Poologasundarampillai, A. Nommeots-Nomm, 3D Printing in Medicine, Materials for 3D printing in medicine: Metals, polymers, ceramics, hydrogels, London: Elsevier, Woodhead Publishing, 2017.

[12] J.-Y. Lee, J. An ,C. K. Chua, *Applied Materials Today*, 2017, 7, 120–133.

[13] G. Sodeifian, S. Ghaseminejada and A. A. Yousefid, Results in Physics, 2019, 12, 205-222,

[14] C. Aumnate, A. Pongwisuthiruchte, P. Pattananuwat and P. Potiyaraj, *Advances in Materials Science and Engineerin*g, 2018, 1-9.

[15] M. Kováčová, J. Kozakovičová, M. Procházka, I. Janigová, M. Vysopal, I. Černičková, J. Krajčovič, Z. Špitalský, *Appl. Sci*., 2020, 10, 9, 3062,

[16] A. Rasooly, A. Weisz, *Antimicrob Agents Chemother*, 2002, 46, 11, 3650–3653.

[17,26] R. Rasooly, *FEMS Immunology and Medical Microbiology*, 2005, 45, 2, 239–244.

[18] N. A. Mir, A. Khan, K. Umar, M. Muneer, *Energy and Environment Focus*, 2013, 2, 208-216.

[19] F. a. D. Administration, Food and Drug Administration,Title 21, Part 74.1328, Washington D.C.: U S Gov ernment Printing Office, 2001, p. 269.

[20] A. d. Groot, J. Nater, J. Weyland, Unwanted Effects of Cosmetics and Drugs Used in Dermatology, Amsterdam: Elsevier, 1994.

[21] J. A. Thies, S. B. Merrill, E. L. Corley, *J Nematol*, 2002, 34, 2, 179-181.

[22] C. E. Steinhart and M. E. Doyle, *Food Safety 1995*, Wisconsin: Marcel Dekker, Inc., 1995, p. 232.

[23] J. D. Bancroft, Theory and Practice of Histological Techniques, London: Elsevier ltd., 2008.

[24] J. G. Waite and A. E. Yousef, *Advances in Applied Microbiology*, USA: Elsevier, 2009, p. 86.

[25] T. J. Lopes, O. H. Gonçalves, R. B. Barros, F. C. R. Capelli, R. A. F. Machado, M. G. N. Quadri and M. B. Quadri, *Brazilian Archives of Biology and Technology*, 2005, 48, 1678-4324.

[27] Q. Wang, C. Zhu, J. Yun and G. Yang, *J. Phys. Chem*. C, 2017, 121, 48, 26722–26732.

# Testicular prothesis in pediatric urology

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***Keywords:*** *Testicular implantation; orchiectomy; testicle transplantation; penis transplantation.*

The absence of a testis from the scrotal sac represents an emotional and psychologically traumatic experience in young males [1]. Although testicular prostheses do not produce hormones or sperm, they can help to complete the normal image of the male body and reduce the level of mental stress in patients with missing testicles [2]. In our conditions, pediatric urology deals with the prevention, diagnosis, and treatment of diseases of the urogenital system in patients from birth to the end of the eighteenth year of life. The testicular absence in the pediatric population is most common after orchiectomy for testicular torsion or trauma, in patients who underwent orchiectomy due to hypoplastic testis in cryptorchidism, or after previous orchidopexy or herniotomy, in patients with unilateral or bilateral anorchia (confirmed by laparoscopic revision of abdominal cavity) and last but not least in patients after radical orchiectomy for testicular tumour. Other indications for orchiectomy such as prostate cancer and gender reassignment surgery are rare in pediatric urology. Implantation of a testicular prosthesis in pediatric patients would require regular replacement because of the appropriate size, that´s why implantation is suitable around the age of 18, which significantly negatively affects the number of applicants in childhood. Although the group of our patients is too small to derive statistically significant results, nevertheless we can observe a positive impact on the quality of life and "self-confidence" of patients. A significant reason for the small number of implanted pediatric patients in Slovakia is the fact that the implantation of a testicular prosthesis is not covered by public health insurance and therefore the patient must pay for the entire procedure from their own resources. It should also be added that patients after the age of 18 may already undergo the procedure at urological department for adults.

## Introduction

Testicular prosthesis placement is a helpful for wide spectrum of pediatric patients. The absence of a testis from the scrotal sac represents an emotional and psychologically traumatic experience in young males. [3] Although testicular prostheses do not produce hormones or sperm, they can help to complete the normal image of the male body and reduce the level of mental stress in patients with missing testicles. [4] In our conditions, pediatric urology deals with the prevention, diagnosis, and treatment of diseases of the urogenital system in patients from birth to the end of the eighteenth year of life.

The testicular absence in the pediatric population is most common after orchiectomy for testicular torsion or trauma, in patients who underwent orchiectomy due to hypoplastic testis in cryptorchidism, or after previous orchidopexy or herniotomy, in patients with unilateral or bilateral anorchia (confirmed by laparoscopic revision of abdominal cavity) and last but not least in patients after radical orchiectomy for testicular tumour. In the future these patients may be interested in testicular implantation for aesthetic or psychological reasons. [5] Other indications for orchiectomy such as prostate cancer and gender reassignment surgery are rare in pediatric urology. Virilizing genitoplasty in patients with disorders of sex development or gender reassignment is another group suitable for testicular implantation.

## Methods

Literature research, used PubMed and Google search with key words: „testicular implantation“, „orchiectomy“, „testicle transplantation“, and „penis transplantation“. Case report of patient with bilateral testicular prothesis implanted at Department of Pediatric Urology, Comenius University, Faculty of Medicine in Bratislava and National Institute of Children's Diseases in Bratislava, Slovakia.

## History

Testicular prostheses produced from various materials such as metal, glass, plastic rubber, polyurethane, and silicone have been in use since 1941. The first testicular implant used in 1941 was made of a chromium-molybdenum cobalt alloy [6]. From 1943, implants were already available in various sizes [7].

After the failure of various materials and techniques evolving in the 1950s the requirements for an ideal prosthesis have been devised which must be chemically and biologically inert, must not cause inflammation, must not be carcinogenic, must withstand mechanical stress, must be sterile, must be able to maintain the desired shape and must be comfortable for the patient. In response to these demands, silicone prostheses have been developed in the 60s of the 20th century. Later, the migration of silicone from silicone-filled testicular, penile, but also breast and other implants into the surrounding tissues confirmed by several authors and also risk of rupture of the implant, involvement of the connective tissue led to a ban on their use and were replaced by new, saline-filled implants [8].

**Risk versus benefits**

The patient's desire to undergo implantation of a testicular prosthesis is a very complex decision [9]. The ratio of possible risks to the expected benefit must always be taken into account. The greatest risks of implantation of testicular prothesis are infection, pain, and discomfort, migration of the prothesis into the inguinal channel or another part of the scrotum, malignancies, and autoimmune diseases of the connective tissue or problems with reproduction. On the other side are long-term psychological benefits of the implants, such as patient satisfaction and improved self-image and psychological outlook [10].

Particular items of dissatisfaction could be: implant too firm (52.4%), shape inconvenient (15.4%), implant too small (23.8%), position too high (30.3%) [11]. Living with a permanent partner had no influence on patient ratings according to Dieckmann. However, Adshead *et al.* observed a lower level of interest in implantation in married men and men living in stable relationships [12]. Over-all satisfaction with implants is high. From 86% to 87,5% of patients would decide again to have a prosthesis [13,14].

Complications related to prosthesis placement may be divided into those related immediately to surgery and those that are later and delayed. Early complications are bleeding, infection, and dehiscence of the surgical wound, described especially in pediatric patients, after surgery performed from the scrotal approach. Complications requiring surgical intervention after implantation of a testicular prosthesis occur in 1,3 – 8% of patients [15,16], but this is not much more than 6% - a complication rate after similar procedures as testicular ablation from the inguinal approach [17]. According to some authors, reoperation after implantation of a testicular prosthe-

sis is in most cases for cosmetic reasons [18]. Late complications are caused by silicone leakage from the implant. There are also references in the literature to spontaneous, unilateral rupture of the testicular prosthesis thirteen years after bilateral implantation [19].

**Materials**

The most frequently used implants are produced in the gel-filled version; however, some companies provide a re-enforced silicone elastomer version called the Soft-Solid Testicular Prosthesis (SSTP). They also provide a saline-filled prosthesis. Prostheses are produced with a suture loop to aid fixation of the implant in the scrotum's most pendant position and reduce unnecessary movement [20]. Prostheses are produced in 5 different sizes.

**Case report**

In a17 - years old male patient monitored due to a syndrome of testicular regression was in one sitting under general anaesthesia performed laparoscopic revision of abdominal cavity (without finding of testicular structures) and bilateral implantation of testicular prothesis from inguinal approach to the upper side of hypoplastic scrotum. Antibiotic prophylaxis was indicated.

Three months after implantation we observed a migration of left prothesis nearly to the inguinal channel. Because of that the position adjustment from scrotal approach under general anaesthesia and antibiotic prophylaxis was indicated. The prothesis was fixed to the bottom of scrotum by two nylon stitches and a glove rubber drain was inserted.

On 13$^{th}$ postoperative day, the patient was acutely readmitted due to large hematoma forming in the left hemi-scrotum and the revision was necessary. During the revision no clear source of active bleeding has been identified. There was found only the small diffuse bleeding areas, which were cauterized. A hemostyptic material (Gelaspon®) was inserted into the wound, the re-fixation of prothesis was performed and the capillary drain was inserted. Operation was performed under antibiotic cover.

Postoperative course was complicated by nearly total wound dehiscence, and the implant material was able to be seen. Removing of the prothesis under general anaesthesia was suggested to the patient, which he did not attend, and he did not come to our clinic again.

## Conclusion

The implantation of a testicular prosthesis of the appropriate size in pediatric patients would require its regular replacement, because of that, its implantation is not suitable until around the age of 18 years, what has a negative impact to number of patients interested in implantation in lower age. Before orchiectomy in pediatric patients, parents have often questions about their subsequent quality of life, psychological impacts and the possibilities of testicular implantation. However, after informing and considering the ratio of possible risks to the expected benefits of the operation, they will not usually decide for implantation in childhood in our conditions. An important reason for the small number of implanted pediatric patients in Slovakia is the fact that the implantation of a testicular prothesis is not covered by public health insurance and therefore the patient must pay the entire procedure from their own resources. It is also important to say, that patients after the age of 18 may already undergo the procedure at the standard urological department for adults.

Although the sample of our patients is too small to derive statistically significant results, we can still observe a positive impact on quality of life and self-image of patients.

Unlike testicular transplantation, which has so far been described in only three patients - identical twins in whom one of the brothers had anorchia, [21] the implantation of a testicular prosthesis does not raise ethical and legal issues such as possible paternity of the child or if to perform a not-lifesaving transplantation and so on [22].

Historically the first described penis and scrotum transplantation performed on March 26, 2018 in a US Armed Forces veteran who suffered a devastating genital injury in Afghanistan also did not include a testicle transplantation [23,24] for ethical reasons, because they would continue to produce donors sperm.

As the long-term effects of "new" foreign materials on the organism sometimes manifests it-self only after decades of use, there is a need for constant creation and testing of new materials.

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## References

[1] J. Money, R. Sollod , *J. Med. Psychol*., **1978**, 51(1),91-4.

[2,4,10] S. Kogan, *Transl Androl Urol.* **2014** ,3(4), 391–397.

[3] J. Money, R. Sollod, *J. Med Psychol*., **1978,** 51(1),91-4.

[5,20] D. J. Bodiwala, T.R. Summerton, *Ann. R. CollSurgEngl,* **2007,** 89(4), 349–353.

[6] J. Girdansky, HF. Newman, *Am J Surg.,* **1941**, 52:514.

[7] C. E. Rea, *J Urol*. **1943**, 49.

[8,9] A. Chapple, A. McPherson, *Psychooncology*, **2004**,13,654–64.

[11,14] K. P. Dieckmann, P.Anheuser, S. Schmidt, B. S. Hundt,U. Pichlmeier, P. Schriefer,C. Matthies, M. Hart mann, C. G. Ruf. *BMC Urol.*, **2015**, 15, 16.

[12] J. Adshead, B, Khoubehi, J. Wood, G. Rustin, *BJU Int.* **2001**, 88, 559–62.

[13] T. G. Clifford, M. L. Burg, B. Hu, J. Loh-Doyle, C. M. Hugen, J. Cai, H. Djaladat, K. Wayne, S. Daneshmand, *Urology*, **2018**, 114,128-132.

[15] L. J. Herrinton, T Brox, S. Greenland, W.D. Finkle, E. Cattolica, S. Shoor, *Ann Epi demiol.*, **2003**,13(1),73–7.

[16] R. Robinson, C. Tait, N. Clarke, V. Ramani, *BJU Int.,* **2014**, 28.

[17,18] P. Anheuser, J. Kranz, J. Will, K.P. Dieckmann, *Urologe A*., **2014**, 53(5), 676–82.

[19] M.S. Floyd Jr, H. Williams, S.K. Agarwal, A.R. De Bolla, *J Med CaseRep.,* **2010**, 26, 4,341.

[21] J. Sherman, J. Silber, *Fertility and Sterility.* **1978,** 30, 2, 181-187.

[22] Available at: https://www.nytimes.com/2019/12/06/health/testicles-transplant.html

[23] Redett R. J., et al., Total Penis, *N Engl J Med.* **2019** Nov 7;381(19):1876-1878

[24] Available at: https://www.hopkinsmedicine.org/news/newsroom/news-releases/johns-hopkins-performs-first-total-penis-and-scrotum-transplant-in-the-world

# Exosomes and cancer cells

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***Keywords:*** *Exosomes, DNA mutations, miRNA.*

Exosomes are small extracellular vesicles that are secreted by various cell types, both healthy and tumorous. MicroRNAs (miRNAs), which are some of the most studied, are found in exosomes protected from degradation. These miRNAs are delivered to target cells, including tumour cells, in which they modulate biological processes. Our understanding of the mechanisms and functions of exosomes has expanded significantly, changing our view of their mechanisms of cell transport and exchange, which underlies the progression of this disease. Cancer cells produce exosomes that have a strong ability to modify the microenvironment around them. This brief review provides information on exosomes and exosomal miRNAs in cancer development, progression, angiogenesis, proliferation, or metastasis. Most importantly, we demonstrate their usefulness in clinical application for cancer patients.

## Exosomes

Among extracellular vesicles, such as apoptotic vesicles, microvesicles, and exosomes, exosomes are the most common group released from mammalian cells [1,2]. Exosomes are small, extracellular, membrane-encapsulated vesicles that range in size from 50 to 150 nm [3]. They are secreted by different cell types: healthy cells and tumour cells. Exosomes are important mediators of cellular communication and regulate various biological processes [4,5].

Exosomes transport proteins and nucleic acids to specific cells. Exosomes transport proteins and nucleic acids to specific cells [6]. Studies show that cancer cells produce 10 times more exosomes than normal, healthy cells. Studies have found that tumour cell-derived exosomes facilitate cellular communication by providing chemokines, growth factors, miRNAs, and others [7,8]. The most interesting of these molecules are miRNAs (**Figure 1.**). They are short, endogenous, non-coding RNAs that regulate gene expression at the post-transcriptional and translational levels [9,10].

Exosomes have their specific expression profiles (mRNA and miRNA), which are different from donor cells [11]. Further studies suggest that cancer exosomal miRNAs play an important role in tumours, as well as the reprogramming of components in the tumour environment [12]. Therefore, exosomal miRNAs are considered potential tools for early diagnostics and therapeutic target. They contain information about signaling pathways that are related to the biological response of the tumour.

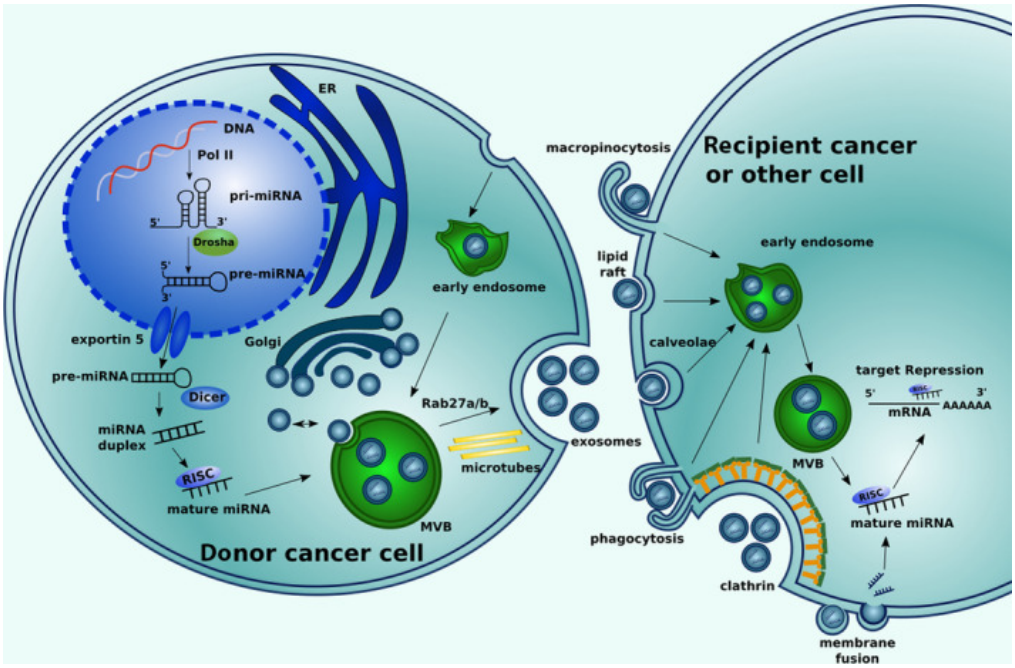
Exosome uptake by recipient cells is processed by endocytosis, receptor-ligand interaction, or fusion to the cell membrane. The uptake of exosomes is not random but depends on the interactions between proteins on the surface of exosomes and recipient cells. Several reports suggest that molecules associated with adhesion to the surface of exosomes, such as tetraspanins, glycoproteins, and integrins, determine which cells accept exosomes [13]. For example, exosomes containing tetraspanin 8 (TSPAN8) and integrin α4 were readily absorbed by CD54 + pancreatic cells [49]. TSPAN8-α4 integrin (CD49d) in exosomes contributed to exosomal binding and uptake by endothelial cells, thereby promoting angiogenesis [14]. Expression of CD47 integrin in engineered exosomes facilitated uptake by tumour cells via micropinocytosis [15].

Exosomes play an important role in intracellular communication, especially during tumour development. Through autocrine and paracrine signalling, they can alter recipient cells. This is done through miRNAs, RNAs, proteins, DNA or metabolites associated with exosomes. An interesting question was whether exosomes contained DNA. This question was posed because exosomes that originate from the multivesicular bodies do not attach to the nucleus. Klluri *et al.* found the presence of double-stranded DNA fragments in exosomes, as well as DNA mutations [16]. The presence of DNA in some exosomes in the stage before their secretion in plasma was detected by transmission electron microscopy [17].

**MiRNA and cancer**

MiRNAs have been found to be closely related to cancer progression, including cancer onset itself, between cancer cells and stromal cells [19,20,21]. Lung cancer accounts for 11.6% of newly reported cancers and 19.8% of all deaths [1]. Its five-year survival rate is one of the lowest among other types of cancer. It is only 18%. Most types of lung cancer are diagnosed at an advanced stage and treatment options are severely limited [22].

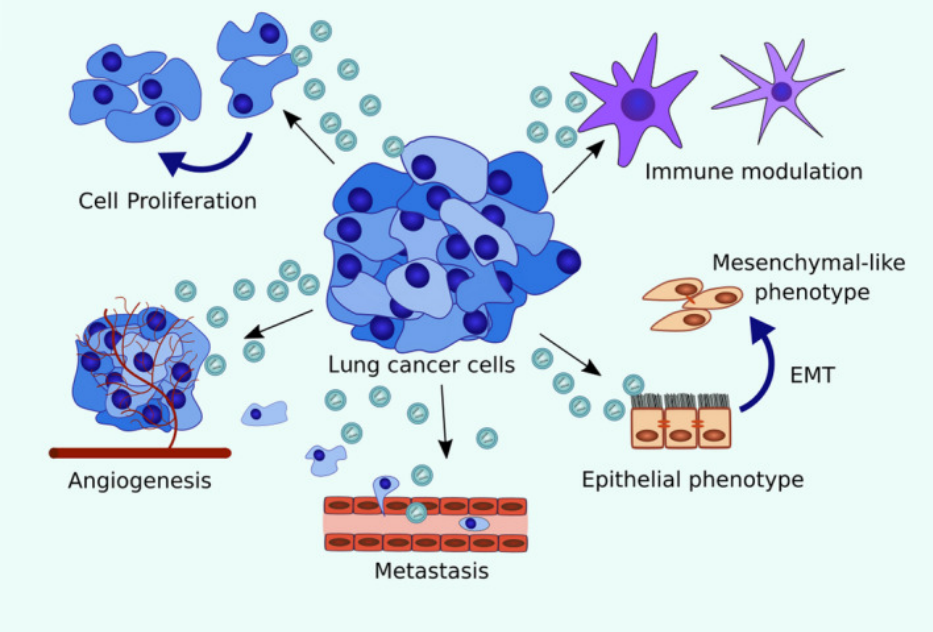
**Figure 1.** Exosomal miRNA. miRNAs are transcribed into primary miRNAs (pri-miRNAs) by RNA polymerase II (Pol-II). Subsequently, they are processed by the Drosha complex to form precursor miRNAs (pre-miRNAs). These are subsequently exported to the cytoplasm by the exportin5 complex, and then cleaved by the Dicer complex into double-stranded miRNAs. They are converted to single-stranded mature miRNAs by helicase. The mature miRNAs are divided into multivesicular bodies (MVBs), and these are then transported to the plasma membrane. When released, we call them exosomes. The exosomes contain a special miRNA from the parent cell and interact with the recipient tumour cell. They interact by fusion through clathrin-dependent endocytosis, clatrin-independent endocytosis: micropinocytosis or phagocytosis, caveolae-mediated endocytosis, or lipid-dependent endocytosis (lipid rafts). When exosomes enter recipient cells, exosomal miRNAs can act towards target repression [18].




In patients with lung cancer, circulating exosome and exosomal miRNA concentrations are elevated compared to appropriate concentrations in control groups [23]. Exosomatic miRNA levels are elevated in plasma and bronchoalveolar lavage samples in patients with non-small cell lung cancer (NSCLC) when compared to non-tumour patients [24]. Recent functional studies have identified relationships between exosomal miRNAs and pathways characteristic of lung cancer from metabolism to intercellular communication (**Figure 2**.). To this end, exosomal miRNAs are involved in many biological processes in lung cancer, including proliferation [25,26], angio-

genesis [27,28,29] and metastases [30,31,32,33]. In addition, exosomal miRNAs affect the lung tumour microenvironment and immune system signalling [34,35,36].

**Figure 2.** Lung cancer and miRNA. Cancer cells transport exosomal miRNAs to the parental cells. There, they affect their angiogenesis, metastasis, proliferation, and epithelial-to-mesenchymal transition (EMT). Cancer cells export exosomal miRNAs to immune cells where they affect their function [18].




**Metastases, proliferation, and angiogenesis**

Exosomes transfer between cancer cells, but also between cancer and stromal cells. Stromal cells take up cancer exosomes, creating a pro-tumour microenvironment. Similarly, cancer cells receive exosomes from stromal cells and facilitate the proliferation or spread of cancer cells [14,37].

Under hypoxic conditions, exosomes from tumour cells may regulate the properties of endothelial cells to promote angiogenesis, while increasing angiogenesis may promote tumour growth [38]. For example, exosomes derived from cancer cells enriched in TSPAN8 and the α4 integrin subunit have increased angiogenesis and endothelial cell proliferation [14]. Hypoxic conditions also stimulated the release of exosomes from glioblastoma. These exosomes increase the regulation of protease-activated receptor 2 (PAR2) in epithelial cells by increasing angiogenesis [39].

Under hypoxic conditions, lung cancer cells produced more miR-23a-enriched exosomes that suppressed their target prolyl hydroxylases 1 and 2 (PHD1 and PHD2). This led to the accumulation of hypoxia-inducible factor-1-alpha (HIF1A) in endothelial cells. Exosomal miR-23a also targeted a linker protein, ZO1, which increased vascular permeability and cancer migration [40].

Similarly, stromal cells affect tumour cells through exosomes. Activated stromal cells located in the vicinity of breast cancer cells release exosomes that contain the cytoplasmic, unshielded RNA RN7SL1. This RNA activates the RIG-1 receptor, which recognizes viral RNA, and this leads to inflammation and tumour progression [41].

There are several studies that suggest that exosomal loads can be used as diagnostic, prognostic, and predictive biomarkers of lung cancer. Exosomal miRNA is the most studied in lung cancer [18].

**Exosomes as a therapeutic tool**

Because exosomes "recognize" specific cells, delivery of therapeutic drugs by exosomes could have an excellent efficacy with precise targeting. Exosomes have therefore become one of the most suitable tools for the delivery and transport of drugs, miRNAs, low interference RNAs (siRNAs), short hairpin RNAs (shRNAs), and other compounds. It is important that they remain stable in exosomes [42] and for this, several strategies have emerged to improve the specificity of targeting tumour cells. One such example: Exosomes should be engineered to express target ligands, such as lysosome-associated membrane protein (LAMP2B) and integrin [43]. Another example is exosomes that deliver siRNA and shRNA. The principle is that these RNAs deplete oncogenes and inhibit tumour growth [15].

Because exosomes can deliver or present tumour-derived antigens, exosomes can be involved in cancer vaccination. The principle is that they activate cytotoxic T cells. One example is the DC exosome vaccine. In these clinical studies, no grade 2 toxicity was observed either at or above this level. Therefore, this route of administration of exosomes is safe [44,45,46]. Because exosomes can be derived from cancer cells, they can therefore induce angiogenesis and thus promote metastasis [47].

Another clinical application of exosomes is the depletion of tumour exosomes from the circulatory system, which in turn blocks metastases. The use of inhibitors prevents the collection and release of exosomes from tumour cells [48], possibly shRNA [49] or the elimination of exosomes from the circulation. For this purpose, the ADAPT system is used to selectively capture and remove circulating HER2 exhalates [50], or potential inhibitors (tipifarnib, ketoconazole, neticonazole, triadimenol, stoupazol) could also be used to eliminate exosomes [51].

## Conclusion

Knowledge of exosomes has expanded in recent years. Exosomes have been studied under both physiological and pathological conditions, and several specific features have been discovered. Exosomes have been found to be mediators of intercellular communication, with cells controlling the cargo within the exosomes. Furthermore, exosomes have been found to affect tumour progression and metastasis, and exosomes can induce anti-tumour responses. Exosomes are also used in the preparation of exosome vaccines that are used in clinical trials. They are based on the fact that exosomes carry specific antigens. It is important to expand the knowledge about the mechanisms and functions of exosomes in the formation and progression of tumours in order to make progress in the field of exosomes and their clinical application in positive patients.

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## References:

[1] C. Fitzmaurice, C. Allen, Global Burden of Disease Cancer C. Global, *JAMA Oncol.*, **2017**, 3, 4, 524-548.

[2] W. Pao, N. Girard, *Lancet Oncol.*, **2011**, 12, 2, 175-180.

[3] C. Thery, M. Ostrowski, E. Segura, *Nat. Rev. Immunol.*, **2009**, 9, 581–593, DOI: 10.1038/nri2567.

[4] J. Chen, C. Hu, P. Pan, *Front Physiol.*, **2017**, 8, 1028.

[5] J. Jabalee, R. Towle, C. Garnis, *Cells*, **2018**, 7, 8, 93.

[6] J. Behera, N. Tyagi, *Oncoscience*, **2018**, 5, 5-6, 181-195.

[7] L. Mao, X. Li, S. Gong, *Cancer Gene Therapy*, **2018**, 25, 9-10, 248-259.

[8] J. C. Akers, D. Gonda, R. Kim, B. S. Carter, C. C. Chen, *J. Neurooncol.*, **2013**, 113, 1, 1-11.

[9] J. Jabalee, R. Towle, C. Garnis, *Cells*, **2018**, 7, 8, 93.

[10] J. Zhang, S. Li, L. Li, **2015**, 13, 1, 17-24.

[11] N. Chaput, C. Thery, *Semin. Immunopathol.*, **2011**, 33, 5, 419-440.

[12] M. Tkach, C. Thery, *Cells*, **2016**, 164, 6, 1226-1232.

[13] S. Rana, S. Yue, D. Stadel, M. Zoller, *The international journal of biochemistry and cell biology*, **2012**, 44, 1574-1584, DOI: 10.1016/j.biocel.2012.06.018.

[14] I. Nazarenko, S. Rana, A. Baumann, J. McAlear, A. Hellwig, M. Trendelenburg, G. Lochnit, K. T. Preissner, M. Zoller, *Cancer Res.*, **2010**, 70, 1668-1678, DOI: 10.1158/0008-5472.CAN-09-2470.

[15] S. Kamerkar, V. S. LeBleu, H. Sugimoto, S. Yang, C. F. Ruivo, S. A. Melo, J. J. Lee, R. Kalluri, *Nature*, **2017**, 546, 498-503, DOI: 10.1038/nature22341.

[16] R. Kalluri, V. S. LeBleu, *Cold Spring Harb Symp Quant Biol*, **2016**, 81, 275–280, DOI: 10.1101/sqb.2016.81.030932.

[17] A. Takahashi, R. Okada, K. Nagao, Y. Kawamata, A. Hanyu, S. Yoshimoto, M. Takasugi, S. Watanabe, M. T. Kanemaki, C. Obuse, E. Hara, *Nat. Commun*, **2017**, 8, 15287, DOI: 10.1038/ncomms15287.

[18] H. Chengping, S. Meiners, Ch. Lukas, G. T. Stathopoulos, J. Chen, *Cell Prolif.*, **2020**, 53, 6, 12828, DOI: 10.1111/cpr.12828.

[19] X. Zhang, B. Sai, F. Wang, *Mol Cancer*, **2019**, 18, 1, 40.

[20] Y. Liu, F. Luo, B. Wang, *Cancer Lett.*, **2016**, 370, 1, 125-135.

[21,22] Z. Xu, X. Liu, H. Wang, *Gene.*, **2018**, 666, 116-122.

[23] G. Rabinowits, C. Gercel-Taylor, J. M. Day, D. D. Taylor, G. H. Kloecker, *Clin. Lung Cancer*, **2009**, 10, 1, 42-46.

[24] M. Rodriguez, J. Silva, A. Lopez-Alfonso, *Genes Chromosomes Cancer*, **2014**, 53, 9, 713-724.

[25] Z. Sun, K. Shi, S. Yang, *Mol. Cancer*, **2018**, 17, 1, 147.

[26] A. Harel, N. Bossel Ben-Moshe, Y. Aylon, *Cell Death Differ.*, **2015**, 22, 8, 1328-1340.

[27] L. Zhang, S. Zhang, J. Yao, *Nature*, **2015**, 527, 7576, 100-104.

[28] H. Cui, B. Seubert, E. Stahl, *Oncogene*, **2015**, 34, 28, 3640-3650.

[29] Y. L. Hsu, J. Y. Hung, W. A. Chang, *Oncogene.*, **2017**, 36, 34, 4929-4942.

[30] D. W. Greening, S. K. Gopal, R. A Mathias, *Semin Cell Dev Biol.*, **2015**, 40, 60-71.

[31] M. W. Klymkowsky, P. Savagner, *Am. J. Pathol.*, **2009**, 174, 5, 1588-1593.

[32] Y. T. Tang, Y. Y. Huang, J. H. Li, *BMC Genom.*, **2018**, 19, 1, 802.

[33] S. Yu, H. Cao, B. Shen, J. Feng, *Oncotarget*, **2015**, 6, 35, 37151-37168.

[34] M. A. Smolle, F. Prinz, G. A. Calin, M. Pichler, *Mol. Aspects Med.*, **2019**, 70, 117-126.

[35] M. A. Cortez, C. Ivan, D. Valdecanas, *J. Natl. Cancer Inst.*, **2016**, 108, 1, 303.

[36] D. W. Greening, S. K. Gopal, R. Xu, R. J. Simpson, W. Chen, *Semin Cell Dev Biol.*, **2015**, 40, 72-81.

[37] J. Skog, T. Wurdinger, S. van Rijn, D. H. Meijer, L. Gainche, M. Sena-Esteves, W. T. Curry Jr., B. S. Carter, A. M. Krichevsky, X. O. Breakefield, *Nat. Cell Biol.*, **2008**, 10, 1470-1476, DOI: 10.1038/ncb1800.

[38] S. Gesierich, I. Berezovskiy, E. Ryschich, M. Zoller, *Cancer research*, **2006**, 66, 7083-7094, DOI: 10.1158/0008-5472.CAN-06-0391.

[39] K. J. Svensson, P. Kucharzewska, H. C. Christianson, S. Skold, T. Lofstedt, M. C. Johansson, M. Morgelin, J. Bengzon, W. Ruf, M. Belting, *Proc. Natl. Acad. Sci. U.S.A.*, **2011**, 108, 13147-13152, DOI: 10.1073/pnas.1104261108.

[40] YL. Hsu, JY. Hung, W. A. Chang, Y. S. Lin, Y. C. Pan, P. H. Tsai, C. Y. Wu, P. L. Kuo, *Oncogene*, **2017**, 36, 4929-4942, DOI: 10.1038/onc.2017.105.

[41] B. Y. Nabet, Y. Qiu, J. E. Shabason, T. J. Wu, T. Yoon, B. C. Kim, J. L. Benci, A. M. DeMichele, J. Tchou, J. Marcotrigiano, A. J. Minn, *Cell*, **2017**, 170, 352-366, e313, DOI: 10.1016/j.cell.2017.06.031.

[42] K. B. Johnsen, J. M. Gudbergsson, M. N. Skov, L. Pilgaard, T. Moos, M. Duroux, *Biochimica et biophysica acta*, **2014**, 1846, 75-87, DOI: 10.1016/j.bbcan.2014.04.005.

[43] Y. Tian, S. Li, J. Song, T. Ji, M. Zhu, G. J. Anderson, J. Wei, G. Nie, *Biomaterials*, **2014**, 35, 2383-2390, DOI: 10.1016/j.biomaterials.2013.11.083.

[44] M. Bourderioux, T. Nguyen-Khoa, C. Chhuon, L. Jeanson, D. Tondelier, M. Walczak, M. Ollero, S. Bekri, B. Knebelmann, E. Escudier, B. Escudier, A. Edelman, I. C. Guerrera, *Journal of proteome research*, **2015**, 14, 567-577, DOI: 10.1021/pr501003q.

[45] S. Dai, D. Wei, Z. Wu, X. Zhou, X. Wei, H. Huang, G. Li, *Mol. Ther.*, **2008**, 16, 782-790, DOI: 10.1038/mt.2008.1.

[46] B. Escudier, T. Dorval, N. Chaput, F. Andre, M. P. Caby, S. Novault, C. Flament, C. Leboulaire, C. Borg, S. Amigorena, C. Boccaccio, C. Bonnerot, O. Dhellin, M. Movassagh, S. Piperno, C. Robert, V. Serra, N. Valente, J. B. Le Pecq, A. Spatz, O. Lantz, T. Tursz, E. Angevin, L. Zitvogel, *J. Transl. Med.*, **2005**, 3, 10, DOI: 10.1186/1479-5876-3-10.

[47] A. Hoshino, B. Costa-Silva, T. L. Shen, G. Rodrigues, A. Hashimoto, M. Tesic, H. Molina, S. Kohsaka, A. Di Giannatale, S. Ceder, S. Singh, C. Williams, N. Soplop, K. Uryu, L. Pharmer, T. King, L. Bojmar, A. E. Davies, Y. Ararso, T. Zhang, H. Zhang, J. Hernandez, J. M. Weiss, V. D. Dumont-Cole, K. Kramer, L. H. Wexler, A. Narendran, G. K. Schwartz, J. H. Healey, P. Sandstrom, K. J. Labori, E. H. Kure, P. M. Grandgenett, M. A. Hollingsworth, M. de Sousa, S. Kaur, M. Jain, K. Mallya, S. K. Batra, W. R. Jarnagin, M. S.

Brady, O. Fodstad, V. Muller, K. Pantel, A. J. Minn, M. J. Bissell, B. A. Garcia, Y. Kang, V. K. Rajasekhar, C. M. Ghajar, I. Matei, H. Peinado, J. Bromberg, D. Lyden, *Nature*, **2015**, 527, 329-335, DOI: 10.1038/nature15756.

[48] F. Chalmin, S. Ladoire, G. Mignot, J. Vincent, M. Bruchard, J. P. Remy-Martin, W. Boireau, A. Rouleau, B. Simon, D. Lanneau, A. De Thonel, G. Multhoff, A. Hamman, F. Martin, B. Chauffert, E. Solary, L. Zitvogel, C. Garrido, B. Ryffel, C. Borg, L. Apetoh, C. Rebe, F. Ghiringhelli, *J. Clin. Invest.*, **2010**, 120, 457-471, DOI: 10.1172/JCI40483.

[49] M. Ostrowski, N. B. Carmo, S. Krumeich, I. Fanget, G. Raposo, A. Savina, C. F. Moita, K. Schauer, A. N. Hume, R. P. Freitas, B. Goud, P. Benaroch, N. Hacohen, M. Fukuda, C. Desnos, M. C. Seabra, F. Darchen, S. Amigorena, L. F. Moita, C. Thery, *Nat. Cell Biol.*, **2010**, 12 19-30, sup. 11–13, DOI: 10.1038/ncb2000.

[50] A. M. Marleau, C. S. Chen, J. A. Joyce, R. H. Tullis, *J. Transl. Med.*, **2012**, 10, 134, DOI: 10.1186/1479-5876-10-134.

[51] A. Datta, H. Kim, L. McGee, A. E. Johnson, S. Talwar, J. Marugan, N. Southall, X. Hu, M. Lal, D. Mondal, M. Ferrer, *Sci. Rep.*, **2018**, 8, 8161, DOI: 10.1038/s41598-018-26411-7.

# Analytical methods in evaluation of the metabolic conversion of 5-fluorocytosine after gene directed enzyme prodrug therapy

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***Keywords:*** *Prodrug therapy, GDEPT, NMR.*

In last decades, the number of cases of oncological diseases has growing trend. This results in increased interest in field of development and research of new oncological treatments and drugs (chemotherapeutics). The main problems of chemotherapeutic treatment are its non-specific cytotoxic effects and the formation of chemoresistant tumors [1]. One approach to reduce the overall toxic effect of the treatment is to use gene directed enzyme prodrug therapy (GDEPT). This procedure is based on the metabolic conversion of a relatively non-toxic precursor (5-fluorocytosine) to an active chemotherapeutic (5-fluorouracil). GDEPT is a three-step mechanism, where the first step is to clone the gene that encodes the production of the enzyme cytosine deaminase (CD) in a viral vector and subsequently its delivery to tumor cells. In the second step, the gene is transcribed into mRNA and the CD enzyme is formed. In the last step, the 5FC is administered to the patient systematically. Subsequently, 5FC is converted to 5FU inside the cell by formed CD. Because CD expression is specifically targeted to tumor cells, healthy cells are minimally affected. In case of resistance to 5FU formation, the enzyme uracil phosphoribosiltransferase (UPRT) is introduced into the CD/5FC system, which supports further metabolic conversion of 5FU [2].

## Analytical approaches for evaluation of metabolic conversion of 5FC after GDEPT

Due to complexity of samples after GDEPT and rapid degradation of 5FC and its major metabolites 5FU, 5-fluorouridine (5FURD), 5-fluorouridine monophosphate (5FUMP) and

5-fluoro-2-deoxyuridine monophosphate (5FdUMP) from the body, it is necessary to use rapid, sensitive and effective analytical methods for the evaluation of the metabolic conversion.

One of the first analytical methods used to evaluate the metabolic conversion of 5FC after GDEPT was the use of nuclear magnetic resonance (NMR). The use of NMR was conditioned by the content of the fluorine atom in the 5FC molecules and its metabolites. The main advantages of using NMR are the possibility to obtain structural information about the analytes and the possibility of its inclusion before another analytical method due to the fact that the sample is not destroyed. However, NMR is limited by properties such as low sensitivity compared to other analytical methods and the need for time-consuming sample pre-treatment and derivatization to determine and confirm the structure of metabolites [3]. T. Dresselaers *et al.* demonstrated NMR-based approach to monitor the metabolic conversion of 5FC to 5FU in HTC116 colon tumour cells. This study showed that NMR is a suitable tool for non-invasive monitoring of metabolic transformation and for evaluation of treatment procedure [4]. In recent time, the most common approach is to use a combination of high-performance liquid chromatography (HPLC) with mass spectrometric detection (MS). Due to properties such as high sensitivity, selectivity, accuracy, separation efficiency, the ability to obtain structural information, the combination of HPLC-MS has become the dominant technique in evaluating the metabolic conversion of 5FC after the GDEPT procedure. An alternative approach to HPLC-HRMS was demonstrated in K. Serve *et al.* study using a combination of HPLC-UV/Vis techniques. The use of the HPLC-UV/Vis combination has the advantage of lower instrumental and operating costs in comparison with HPLC-MS, but it also has limits in lower sensitivity, detection limits and selectivity [6]. Another alternative approach has been shown in Y. Liu et al. study that monitored the metabolic conversion of 5FC to 5FU using a combination of capillary zone electrophoresis (CZE) techniques with UV/Vis detection. The CZE offers advantages over HPLC such as lower operating cost, low solvent and samples consumption. However, the coupling of CZE to MS is more technically and operationally demanding than HPLC-MS [7]. In our recent study, a combination of HPLC with high-resolution mass spectrometry (HRMS) was used to monitor the metabolic conversion of 5FC to its major metabolites. The metabolic conversion of 5FC to 5FU, as well as to other metabolites in the 5FC/CD and 5FC/CD/UPRT systems, was studied in HT-29 tumor cell samples and mesenchymal stem cells after the GDEPT procedure. An efficient metabolic transformation in chemo-resistant tumour cells was also observed in this study. From the obtained

results it can be concluded that the HPLC-HRMS combination is a suitable tool for the analysis of samples after the GDEPT procedure [5].

## Conclusion

Research in the treatment of oncological diseases is associated with the need to develop more effective analytical methods for evaluating the effectiveness of new treatments. Currently, the most commonly used technique is combination of HPLC-MS, especially HPLC-HRMS. Properties such as low detection limits, high selectivity, and the ability to obtain structural information of analytes allow evaluation of new treatments, which helps with further research and development.

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## References

[1] M. Breda, S. Barratè, *Analytical and Bioanalytical Chemistry* [online], **2010**, 397, 3, 1191-1201, ISSN 16182642. doi:10.1007/s00216-010-3633-8.

[2] J. Zhang, V. Kale, M. Chen, *The AAPS Journal* [online], **2015**, 17, 1, 102-110, ISSN 1550-7416. doi:10.1208/s12248-014-9675-7.

[3] R. Martino, F. Desmoulin, M. Malet-Martino, *Journal of Pharmaceutical and Biomedical Analysis* [online], **2005**, 38, 871-91. doi:10.1016/j.jpba.2005.01.047.

[4] T. Dresselaers, J. Theys, S. Nuyts, B. Wouters, E. de Bruijn, J. Anne, P. Lambin, P. van Hecke, W. Landuyt, *British Journal of Cancer* [online], **2003**, 89, 1796-1801. doi:10.1038/sj.bjc.6601345.

[5] E. Durinikova, J. Plava, S. Tyciakova, P. Skvara, A. Vojs Stanova, Z. Kozovska, L. Kucerova, M. Matuskova, *Cancer Gene Therapy* [online], **2018**, 25, 11-12, 285–299, doi:10.1038/s41417-018-0030-5.

[6] Serve, M. Kinta, J. A. Yáñez, C. M. Remsberg, N. M. Davies, M. E. Black, *Biomedical Chromatography* [online], **2010**, 24, 5, 556-561, ISSN 02693879. doi:10.1002/bmc.1326.

[7] Y. Liu, P. Zhu, Z. Huang, L. Zhou, P. Shi, *Journal of Chromatography B* [online], **2018**, 1076, 1-7, ISSN 1873376X. doi:10.1016/j.jchromb.2018.01.011.

# MicroRNA in lung cancer therapy - focused on non-small cell lung cancer (NSCLC)

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***Keywords:*** *MiRNA, markers.*

Lung cancer is the most diagnosed cancer in the world out of which 80% represents non-small cell lung cancer (NSCLC). Since NSCLC is often diagnosed in advanced stages, which affects patient's survival, reliable biomarkers of early stages are needed. MicroRNAs (miRNAs) are small noncoding RNAs functioning as posttranscriptional regulators of gene expression. They play an important role in cell proliferation, apoptosis, differentiation or drug resistance and their dysregulation is connected to diseases or cancer state. MiRNAs have been identified in multiple body fluids, including plasma and serum, as cell-free or circulating miRNAs. Distinct miRNA expression profiles were observed in specific malignancies; therefore, these miRNAs can serve in diagnostics as non-invasive predictive biomarkers and thus they have great potential in personalized medicine. This brief review summarizes current studies of miRNA involvement in NSCLC, its diagnostic potential and therapeutic strategies targeting microRNA in lung cancer and mentions the only miRNA-based therapeutic on phase 1 clinical trial – MRX34.

## Introduction

According to Global cancer statistics (2018), lung cancer (LC) remains the most diagnosed cancer with 11.6 % of all the cancer cases and it also remains the leading cause of death (18.4 %) caused by cancer [1]. There are two main types of LC – small cell lung cancer (SCLC) which represents 20 % of the LC and non-small cell lung cancer (NSCLC) representing 80 % of the LC cases. The latter comprise of three main histological types – adenocarcinoma, squamous-cell carcinoma, and large cell carcinoma [2]. Squamous-cell carcinoma and SCLC are mainly diagnosed in tobacco smoking-connected LC, whilst non-smokers and women are mostly diagnosed with the adenocarcinoma [3]. SCLC is mostly aggressive with rapid doubling time and

high rate of dissemination. Nevertheless, SCLC possess increased sensitivity to radio- and chemotherapy [4]. NSCLC, on the other hand, has quiet slower onset but NSCLC cells are more resistant to chemo- or radiotherapy. NSCLC is often diagnosed in advanced stage of the disease and the survival rate of patient remains poor – 68% patients with stage IB and 0-10% patients with stage IVA-IVB survive next 5 years [5]. The possible cause of late diagnoses of NSCLC might be a lack of reliable biomarkers, which would indicate lung cancer in an early stage when the tumour is still resectable [6]. Treatment of NSCLC is stage specific. In the early stages, considering insufficient response to radio- or chemotherapy, at the first place there is an attempt for complete surgical resection supplemented by adjuvant chemotherapy. In palliative treatment, chemotherapy, radiotherapy, and targeted therapy are used. The targeted therapy focuses on vascular endothelial growth factor or its receptor, or epidermal growth factor (EGF) and its receptor (EGFR) [7].

In formation and onset of the LC, alterations in tumour-suppressor and protooncogenes arise. Apart of mutations in tumour-suppressors and protooncogenes, in LC chromosomal aberrations are common. Interestingly, near the chromosomal fragile sites, common breakpoints involved in cancer, are localized circa half of microRNA (miRNAs) genes [8].

**MiRNA**

MicroRNAs are small (19-23) single stranded RNAs, first time identified in 1993 by Ambros group while studying temporal control of diverse postembryonic development in Caenorhabditis elegans [9]. MiRNAs function as small noncoding posttranscriptional regulators of gene expression which happens according to their complementarity with “seed” sequence at the 3’ or 5’ UTR of the target mRNA. Biogenesis of miRNA is divided to canonical and non-canonical pathway. In the first one, transcription of miRNA genes by RNA polymerase II results in pri-miRNA. Primary miRNA is processed by RNAseIII Drosha and its cofactor DGCR8 (DiGeorge Syndrome Critical Region 8) into pre-miRNA [10]. This processing leaves two-nucleotides long overhangs at the 3’ end, which are recognized, and the miRNA is exported to the cytoplasm via exportin 5 (Xpo5): RanGTP [11]. In the cytoplasm the precursor miRNA is processed by RNaseIII Dicer and its cofactor TRBP: PACT protein. The cleavage results in miRNA:miRNA* duplex [12]. Non-canonical processing of miRNA transcripts can be divided into Drosha and DGCR8-independent or Dicer-independent pathways. The difference can also occur in the process of exporting the nucleus, where exportin 1 is alternatively involved [13,14]. Thermodynam-

ic stability of 5'ends or presence of U at nucleotide position 1 at 5'end determines leading (miRNA) and so-called passenger strand (miRNA*) in the miRNA: miRNA* duplex [15]. The passenger strand is degraded, and the leading strand is associated with Argonaute proteins Ago1 and Ago2 which are the main components of the RNA-induced silencing complex (RISC). The RISC subsequently recognizes and binds to 3' or 5' UTR of the target mRNA according to complementarity with miRNA, which results either in translational repression or mRNA deadenylation. As already reviewed by Vasuvedan [16], miRNA can also upregulate expression both directly by miRNP-mediated translational activation and indirectly in the process of relief of repression which was described in muscle differentiation regulated via miR-145 [17].

**MiRNA as diagnostic markers**

Due to miRNA function as posttranscriptional regulators, they are involved in diverse biological processes, such as cell proliferation, development, or apoptosis. Therefore, their dysregulation leads to disease states and cancer. Difference in miRNA expression profiles in patients with NSCLC, NSCLC cell lines and healthy control has been reported. In the study of Raponi *et al.,* comparison of 61 patients with squamous-cell carcinoma and 10 healthy lung controls revealed 15 differentially expressed miRNAs. The majority was highly expressed in tumour cells including members of miR-17-92 cluster, its paralogue clusters miR-106a-363 and miR-93-106b and members of miR-182-183 cluster. Down-regulated were miRNAs from the miR-125a-let7e cluster [18]. The members of let7 family of miRNAs function as tumour-suppressors [19,20,21]. In another study, 20 fine-needle aspiration samples were analysed. In tumoral samples miR-21 (in 65% of samples), miR-155 (50% of samples) and miR-7 (60% of samples) showed higher expression and let7a lower expression levels (in 60% of samples) than healthy samples. Interestingly samples with EGFR-activating mutations in exons 19 and 21 showed more than 2-fold higher expression of miR-21 and miR-7 [22]. Zhang et al. investigated diverse miRNA expression in 51 adenocarcinoma (AD) samples comparing to tissue samples of 54 squamous-cell carcinoma (SCC). Compared to non-neoplastic lung tissue, AD samples had significantly decreased levels of miR-125-5p and let-7e; and SCC tissues had elevated levels of miR-93, miR-205 and miR-221 and decreased levels of miR-125a-5p and let-7e. Comparing SCC samples to AD samples, SCC showed increased expression levels of miR-205, miR-221 and miR-30e, while the levels of miR-29b, miR 125a 5p and let-7e were decreased. Interestingly miR-29c, miR-93 and miR-100 showed no significant difference between these malignancies, therefore according to expression profiles of the earlier mentioned miRNAs we can distinguish between AD and SCC

[23]. Meta-analysis of 14 miRNA profiling studies that compared cancer tissues with normal tissues revealed four highly expressed (miR-210, miR-21, miR-31, miR-182) and two downregulated miRNAs (miR-126 and miR-145) in SCC and AD with other 14 miRNAs reported either in one or another [24].

Since previous studies mentioned analyses of miRNAs profiles from tissue and as was already mentioned, some LC patients cannot be operable, biomarkers detectable from blood sample are required. MiRNAs in stable form protecting them from endogenous RNase activity were identified in human plasma in 2008. In this study Mitchells group observed that miRNAs from human prostate cancer xenografts entered mice circulation and could be readily measured in plasma [25]. Two years later miRNAs were detected in all the body fluids including saliva, tears, urine, or sputum [26]. To investigate whether candidate circulating miRNAs could be used as biomarkers in early stages of NSCLC, Fan et al. analyzed 94 NSCLC patients serum samples and 58 healthy serum samples by qRT PCR and to support these results they analysed another 70 NSCLC patients and 54 healthy controls sera by florescence quantum dots liquid bead array. Out of 12 candidate miRNAs five (miR-16-5p, miR-17b-5p, miR-19-3p, miR-20a-5p, miR-92-3p) were significantly downregulated and miR-15b-5p was significantly upregulated in NSCLC samples. Predictive model showed that miR-15b-5p, miR-16-5p and miR-20a-5p can distinguish healthy samples from NSCLC [27]. Later, a diagnostic test to identify asymptomatic high-risk individuals with early stage of lung cancer by analysing expression profiles of 34 miRNAs was published. This test of serum miRNAs can identify asymptomatic high-risk individuals with 80% accuracy [28]. Results of meta-analysis by Chen and Jin support incorporating miRNAs assay to initial NSCLC screening before using computed tomography or any other imaging methods thanks to its relatively high sensitivity (76%) and specificity (80%) [29]. Early diagnostics and proper design of treatment are key in patient's survival, therefore identifying prognostic miRNAs has a great importance. Sanfiorenzo *et al.* compared miRNAs levels in plasma of 52 NSCLC patients with I-IIIA stages, 10 patients with chronic obstructive pulmonary disease and 20 healthy individuals. In this study three miRNA plasma signature (high miR-155-5p, high miR-223-3p, and low miR-126-3p) significantly associated with higher risk for progression in AD patients and three plasma signature (high miR-20a-5p, low miR-152-3p, and low miR-199a-5p) predicting survival of SCC patients were observed [30]. Other prognostic miRNAs are miR-98-5p, mir-302e, miR-495-3p and miR-613 with higher expression in patients with com-

plete or partial recovery after radiotherapy compared to patients with stable or progressive disease after radiotherapy [31].

**MiRNA therapeutic strategies**

Up to this day multiple miRNA expression profiles connected to specific malignancies were published [32,33,34,35]. Knowledge of patient's miRNA expression profile can help to tailor exact treatment the patient needs, therefore potentially improving his survival. In this kind of treatment fine-tuning levels of target miRNAs is necessary. One of the approaches is preparation of anti-miRNA oligonucleotides (AMO) which bind either target mRNA, pre-miRNA, or mature miRNA, therefore blocking their activity. AMO undergoes chemical modifications of the sugar, base, or inter-nucleotide linkage, which improve their binding affinity, delivery, and their resistance against nucleases [36]. In NSCLC were AMO used in functional studies of miRNAs. Overexpression of miR-374b reduced viability of NSCLC cells (in tissue collected from patients) by promoting apoptosis and inhibiting formation of tumours. On the contrary overexpression of AMO corresponding to miR374b led to increased colony formation by NSCLC. Another loss-of-function approach targeting oncomiRs (oncogenic mRNAs) is the use of miRNA sponges, RNA molecules containing in tandem antisense sequence of target miRNA. Binding to miRNA sponge prevents miRNAs from binding to their endogenous target, thus the mRNA proceeds with translation [37]. Interestingly, sponge-like activity of circular RNAs (circRNA) has been recently observed. CircRNAs are functional molecules of RNA circularized thru typical 5' to 3'-phosphodiester bonds, which arise from the process of backsplicing [38]. CircRNAs act like sponges for miRNAs, RBPs and can compete with linear splicing. Dysregulation of circRNA expression leads to disease development [39]. Overexpression of circRNA circHIPK3 in NSCLC cell lines led to promotion of the cell proliferation while its knock-down inhibited the cell proliferation. CircHIPK3 acts as sponge for both miR-379 and its target insulin-like growth factor 1 mRNA, therefore suggesting tumour-suppressor role of miR 379 [40]. This miRNA with whole cluster miR 379/miR-656 is downregulated in glioblastoma, kidney renal clear cell carcinoma, breast invasive carcinoma and ovarian cystadenocarcinoma [41]. Further knowledge on circRNA is still needed, but in the future their sponging activity might be an interesting target in cancer therapy. Antisense oligonucleotides with perfect complementarity to predicted miRNA-binding site on target mRNAs (to seed sequence) are called miR-MASKs. These oligonucleotides were for the first time prepared to study Zebra fish miR-340/mRNA interaction by masking the binding site for miRNA therefore blocking its action [42]. Mir-MASKs helped to

explore the role of miR-522 in NSCLC. In this study both NSCLC cell lines and patient's tissues samples contained elevated miR-522 levels and adding a miR-522 inhibitor led to reduced migration of cells and invasion. Furthermore, application of miR-MASK complementary to potential target miR-522 – mRNA DENN/MADD domain containing 2D (DENND2D) suppressed NSCLC cell proliferation and induced apoptosis [43].

In the therapy where upregulation of tumour-suppressor miRNAs is needed so-called miRNA mimics could be prepared. The first potential miRNA-based therapeutic, MRX34, is based on miRNA mimics of miR 34, naturally occurring tumour-suppressor, which is lost or has low expression levels in cancer cells [44]. Members of miR-34 family inhibit cell proliferation and tumour-cell invasion, and negatively regulate epithelial-mesenchymal transition [45]. The MRX-34 therapeutic is based on liposomal nanoparticles containing the miR-34 mimics. When introduced to cancer cells, miR-34a - which functions directly in p53 pathway – activates cell-cycle arrest and apoptosis [46, 47]. This therapeutic originally presented as potential therapeutic for liver cancer, is now on phase 1 clinical trial (NCT01829971). However, this trial had to be closed early due to immune-mediated adverse events which resulted in death of four patients [48].

**Conclusion**

Cancer incidence and cancer-caused mortality are worldwide growing. The most diagnosed cancer remains LC out of which NSCLC represents most of the cases. Late diagnostics often in advanced stages of the disease, worsen patient's prognosis of survival. Therefore, reliable non-invasive biomarker is needed. Circulating miRNAs, small posttranscriptional regulators of gene expression commonly dysregulated in cancer, present in body fluids including serum and plasma showed great potential as such biomarkers [27,28,29,30,31]. Expression profiles of not just one but rather a panel of miRNAs can help to diagnose high-risk individuals in early even asymptomatic stages of NSCLC [28]. MiRNA expression profiles from tissue samples can distinguish SCC from AD [23] and circulating miRNAs can serve as prognostic markers in SCC and AD [30]. Furthermore, miRNAs expression profiles can also predict how will patients respond to radiotherapy [31]. Early diagnosis with exact knowledge of patient's malignancy, stage, prognosis, and potential prediction of response to therapy are key factors in personalized medicine and could have a great impact on patient's survival. In addition, expression profiling of miRNAs suggests potential therapeutic targets. Multiple strategies for oncomiR down-regulation, such as

AMOs, miRNA-sponges, or miR-MASKs, had been characterized. Interestingly, circRNAs possess sponging activity [38, 39, 40], therefore they may serve as an alternative therapeutic target in oncomiRs down-regulation. Since crircRNAs sponge not only miRNAs, but also mRNAs and RBPs, further research is needed in this field [38, 40]. On the contrary of oncomiRs, up-regulation of tumour-suppressor miRNAs has gone further in the therapeutic field. Liposomic form of miR-34a mimics is the first potential miRNA-based therapeutic. Even thou MRX therapeutic currently had to close early its clinical trial, an idea of miRNA-based therapeutics can broaden patients' options [48]. In such complex and developing disease as cancer we need to stress an importance of translational research. Identification of miRNA expression profiles characteristic to cancer stage or suggesting prognosis of the patient may play an important role in diagnostics and further patient's treatment.

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**References**

[1] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, A. Jemal, CA, *A Cancer Journal for Clinicians*, **2018**, 68, 6, 394-424.

[2] W. D. Travis, E. Brambilla, A. G. Nicholson, Y. Yatabe, J. H. M. Austin, M. B. Beasley, L. R. Chirieac, S. Dacic, E. Duhig, D. B. Flieder, K. Geisinger, F. R. Hirsch, Y. Ishikawa, K. M. Kerr, M. Noguchi, G. Pelosi, C. A. Powell, M. S. Tsao, I. Wistuba, WHO Panel, *Journal of Thoracic Oncology*, **2015**, 10, 9, 1243-1260.

[3] N. D. Freedman, C. C. Abnet, N. E. Caporaso, J. F. Fraumeni, G. Murphy, P. Hartge, A. R. Hollenbeck, Y. Park, M. S. Shiels, D. T. Silverman, *International Journal in Epidemiology*, **2016**, 45, 3, 846-856.

[4] E. Rodriguez, R. C. Lilenbaum, *Current Oncology Reports*, **2010**, 12, 327-334.

[5] P. Goldstraw, K. Chansky, J. Crowley, R. Rami-Porta, H. Asamura, W. E. E. Eberhardt, A. G. Nicholson, P. Groome, A. Mitchell, V. Bolejack, *Journal of Thoracic Oncology*, **2016**, 11, 1, 39-51.

[6] J. Hou, F. Meng, L. W. C. Chan, W. C. S. Cho, S. C. C. Wong, *Frontiers in Genetics*, **2016**, 7-193, DOI: 10.3389/fgene.2016.00193.

[7] N. Duma, R. Santana-Davila, J. R. Molina, *Mayo Clinic Proceedings*, 94, 8, 1623-1640.

[8] G. A. Calin, C. Sevignani, C. D. Dumitru, T. Hyslop, E. Noch, S. Yendamuri, M. Shimizu, S. Rattan, F. Bullrich, M. Negrini, C. M. Croce, *PNAS*, **2003**, 101, 9, 2999-3004.

[9] R. C. Lee, R. L. Fenbaum, V. Ambros, *Cell*, **1993**, 75, 5, 843-854.

[10] A. M. Denli, B. B. J. Tops, R. H. A. Plasterk, R. F. Ketting, G. J. Hannon, *Nature*, **2004**, 432, 231-235.

[11] R. Yi, Y. Qin, I. G. Macara, B. R. Cullen, *Genes and Development*, **2003**, 17, 24, 3011-3016.

[12] Y. Lee, I. Hur, S.-Y. Park, Y.-K. Kim, M. R. Suh, V. N. Kim, *EMBO Journal*, **2006**, 25, 3, 522-532.

[13] E. Berezikov, W.-J. Chung, J. Willis, E. Cuppen, E. C. Laj, *Molecular Cell*, **2007**, 28, 2, 328-336.

[14] E. Herrera-Carrillo, B. Berkhout, *Nucleic Acid Research*, **2017**, 45, 18, 10369-10379.

[15] A. Khvorova, A. Reynolds, S. D. Jayasena, *Cell*, **2003**, 115, 209-216.

[16] S. Vasudevan, *WIREs RNA*, **2012**, 3, 311-330.

[17] K. R. Cordes, N. T. Sheehy, M. White, E. Berry, S. U. Morton, A. N. Muth, T.-H. Lee, J. M. Miano, K. N. Ivey, D. Srivasta, *Nature*, **2009**, 460, 7256, 705-710.

[18] M. Raponi, L. Dossey, T. Jatkoe, X. Wu, G. Chen, H. Fan, D. G. Beer, *Cancer Research*, **2009**, 69, 14, 5776-5783.

[19] S. M. Johnson, H. Grosshans, J. Shingara, M. Byrom, R. Jarvis, A. Cheng, E. Labourier, K. L. Reinert, D. Brown, F. J. Slack, *Cell*, **2005**, 120, 5, 635-647.

[20] V. B. Sampson, N. H. Rong, J. Han, Q. Yang, V. Aris, P. Soteropoulos, N. J. Petrelli, S. P. Dunn, L. J. Krueger, *Cancer Research*, **2007**, 67, 20, 9762-9770.

[21] Y. S. Lee, A. Dutta, *Genes and Development*, **2007**, 21, 9,1025-1030.

[22] D. Petriella, D. Galetta, V. Rubini, E. Savino, A. Paradiso, G. Simone, S. Tommasi, *Molecular Biotechnology*, **2013**, 54, 913-919.

[23] Y.-K. Zhang, W.-Y. Zhu, J.Y. He, D.-D. Chen, Y.-Y.Huang, B.-H. Le, X.-G. Liu, *Journal of Cancer Research and Clinical Oncology*, **2012**, 138, 1641-1650.

[24] P. Guan, Z. Yin, X. Li, W. Wu, B. Zhou, *Journal of Experimental and Clinical Cancer Research*, **2012**, 31, 54, DOI:10.1186/1756-9966-31-54.

[25] P. S. Mitchell, R. K. Parkin, E. M. Kroh, B. R. Fritz, S. K. Wyman, E. L. Pogosova-Agadjanyan, A. Peterson, J. Noteboom, K. C. O'Briant, A. Allen, D. W. Lin, N. Urban, C. W. Drescheer, B. S. Knudsen, D. L. Stirewalt, R. Gentleman, R. L. Vessella, P. S. Nelson, D. B. Martin, M. Tewari, *PNAS*, **2008**, 105, 30, 10513-10518.

[26] J. A. Weber, D. H. Baxter, S. Zhang, D. Y. Huang, K. H. Huang, M. J. Lee, D. J. Galas, K. Wang, *Clinical Chemistry*, **2010**, 56, 11, 1733-1741.

[27] L. Fan, H. Qi, J. Teng, B. Su, H. Chen, C. Wang, Q. Xia, *Tumor Biology*, **2016**, 37, 7777–7784.

[28] F. Bianchi, F. Nicassio, M. Marzi, E. Belloni, V. Dall'Olio, L. Bernard, G. Pelosi, P. Maisonneuve, G. Veronesi, P. P. Di Fiore, *EMBO Molecular Medicine*, **2011**, 3, 9, 495-503.

[29] L. Chen, H. Jin, *Tumor Biology*, **2014**, 35, 9119-9129.

[30] C. Sanfiorenzo, M. I. Illie, A. Belaid, F. Barlési, J. Mouroux, C.-H. Marquette, P. Brest, P. Hofman, *PLoS One*, **2013**, 8, 1, https://doi.org/10.1371/journal.pone.0054596.

[31] X. Chen, Y. Xu, X. Liao, R. Liao, L. Zhang, K. Niu, D. Li, Z. Chen, Y. Duan, J. Sun, *Tumour Biology*, **2016**, 37, 9, 11927-11936.

[32] D. L. Zanette, F. Rivadavia, G. A. Molfetta, F. G. Barbuzano, R. Proto-Siqueira, R. P. Falcao, M. A. Zago, W. A. Silva-Jr., *Brazilian Journal of Medical and Biological Research*, **2007**, 40, 11, 1435-1440.

[33] R. Søkilde, H. Persson, A. Ehinger, A. C. Pirona, M. Fernö, C. Hegardt, C. Larsson, N. Loman, M. Malmberg, L. Rydén, L. Saal, Å. Borg, J. Vallon-Christerson, C. Rovira, *BMC Genomics*, **2019**, 20, 503, https://doi.org/10.1186/s12864-019-5887-7.

[34] A. Piepoli, F. Tavano, M. Copetti, T. Mazza, O. Palumbo, A. Panza, F. F. di Mola, V. Pazienza, G. Mazzoccoli, G. Biscaglia, A. Gentile, N. Mastrodonato, M. Carella, F. Pellegrini, P. di Sebastiano, A. Andriulli, *PLoS ONE*, **2012**, 7, 3, https://doi.org/10.1371/journal.pone.0033663.

[35] M. Slattery, J. S. Herrick, L. E. Mullany, E. Wolff, M. D. Hoffman, D. F. Pellatt, J. R. Stevens, R. K. Wolff, *Modern Pathology*, **2016**, 29, 915-927.

[36] U. Moens, *Journal of Biomedicine and Biotechnology*, **2009**, 2009, DOI:10.1155/2009/419539.

[37] M. S. Ebert, J. R. Neilson, P. S. Sharp, *Nature methods*, 4, 9, 721-726.

[38] W. R. Jeck, J. A. Sorrentino, K. Wang, M. K. Slevin, C. E. Burd, J. Liu, Marzluff, N. E. Sharpless, *RNA*, **2013**, 19, 2, 141-157.

[39] K.-Y. Hsiao, H. S. Sun, S.-J. Tsai, *Experimental Biology and Medicine*, **2017**, 242, 1136-1141.

[40] F. Tian, Y. Wang, Z. Xiao, X. Zhu, *Chinese Journal of Lung Cancer*, **2017**, 20, 7, 459-467.

[41] S. V. Laddha, Nayak, N. Subhashree, D. Paul, R. Reddy, C. Sharma, P. Jha, M. Hariharan, A. Agrawal, S. Chowdhury, C. Sarkar, A. Mukhopadhyay, *Biology Direct*, **2013**, 10, https://doi.org/10.1186/1745-6150-8-10.

[42] W.-Y. Choi, A. J. Giraldez, A. F. Schier, *Science*, **2007**, 318, 271-274.

[43] T. Zhang, Y. Hu, J. Ju, L. Hou, Z. Li, D. Xiao, Y. Li, J. Yao, C. Wang, Y. Zhang, L. Zhang, *Scientific Reports*, **2016**, 6, https://doi.org/10.1038/srep19346.

[44] H. Hermeking, *Cell Death and Differentiation*, **2010**, 17, 193-199.

[45] L. Zhang, Y. Liao, L. Tang, *Journal of Experimental & Clinical Cancer Research*, **2019**, 38, 53, https://doi.org/10.1186/s13046-019-1059-5.

[46] V. Tarasov, P. Jung, B. Verdoodt, D. Lodygin, A. Epanchintsev, A. Menssen, G. Meister, H. Hermeking, *Cell Cycle*, **2007**, 6, 13, 1586-1593.

[47] H. Tazawa, N. Tsuchiya, M. Izumiya, H. Nakagama, *PNAS*, **2007**, 104, 39, 15472-15477.

[48] D. S. Hong, Y.-K. Kang, M. Borad, J. Sachdev, S. Ejadi, H. Y. Lim, A. J. Brenner, K. Park, J.-L. Lee, T.-Y. Kim, S. Shin, C. R. Becerra, G. Falchook, J. Stoudemire, D. Martin, K. Kelnar, H. Peltier, V. Bonato, A. G. Bader, S. Smith, S. Kim, V. O'Neil, M. S. Beg, *British Journal of Cancer*, **2020**, 122, 1630-1637.

# Tetra(trimethylgalloyl)oxyferuloyl quercetin: protective effect on beta cell viability decrease induced by methylglyoxal in the pancreatic INS-1E tumour cell line

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This work is aimed at the study of pancreatic INS-1E tumour beta cell line impairment induced by the glucose metabolite, metylglyoxal, and modulatory effect of quercetin derivative, tetra(trimethylgalloyl)oxyferuloyl quercetin, on beta cell viability.

We found that this quercetin derivative significantly increased the viability of beta cells and showed protective effect on cell viability decrease induced by methylglyoxal in the pancreatic INS-1E cell line. The protective effect of this derivative may be attributed to the presence of ferulic and gallic acid, owing to their strong antioxidant properties.

## Introduction

Type 2 diabetes is a complex metabolic disorder. It is associated with insulin resistance (IR), impaired insulin signalling, beta-cell dysfunction, abnormal glucose levels and altered lipid metabolism. It is also linked to sub-clinical inflammation and increased oxidative stress (Testa et al., 2016). Pancreatic beta cells are highly susceptible to oxidative and ER stresses. One reason may be that islets of Langerhans contain among the lowest levels of antioxidant enzyme activities compared to other tissues (Robertson & Harmon, 2006). One of the main causes of oxidative stress in pancreatic beta cells is glucotoxicity. Methylglyoxal (MGX) plays an important role in glucotoxicity. MGX is a spontaneous product of glucose metabolism which is known to cause cytotoxic actions and to be present in raised concentrations in hyperglycaemia (Sheader *et al.,* 2001). Compounds with antioxidant properties and free radical scavengers may

help in the regeneration of beta cells and protect pancreatic cells against the cytotoxic effects of MGX. Quercetin, a member of the flavonoid family, is one of the most prominent dietary antioxidants. Quercetin was reported to trap MGX and then significantly inhibits the formation of AGEs (Li *et all.,* 2014). The antioxidant activity of certain natural products and their analogues can be enhanced by synthesizing new derivatives based on active pharmacophore models, drug resistance and solubility and metabolic limitations can be overcome by appropriate molecular modifications and new biological properties or mechanisms of action can be added by combining other functional groups or molecules.

## Methods

### Cell culture

INS-1E pancreatic cell line (kindly provided by Prof. Claes Wollheim, University of Geneva) was cultured in RPMI 1640 medium containing 11 mM glucose (Sigma Aldrich) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, 1 mM Na-pyruvate, 55 µM 2-mercaptoethanol, 10 mM HEPES, non-essential amino acid (1%) and 10% fetal calf serum, pH 7.0-7.4. The maintenance culture was passaged once a week by gentle trypsinization (7 min). Cells were seeded at a density of $5\times10^6$ cells in 75-$cm^2$ Falcon bottles in 17 ml complete medium.

### Cell viability

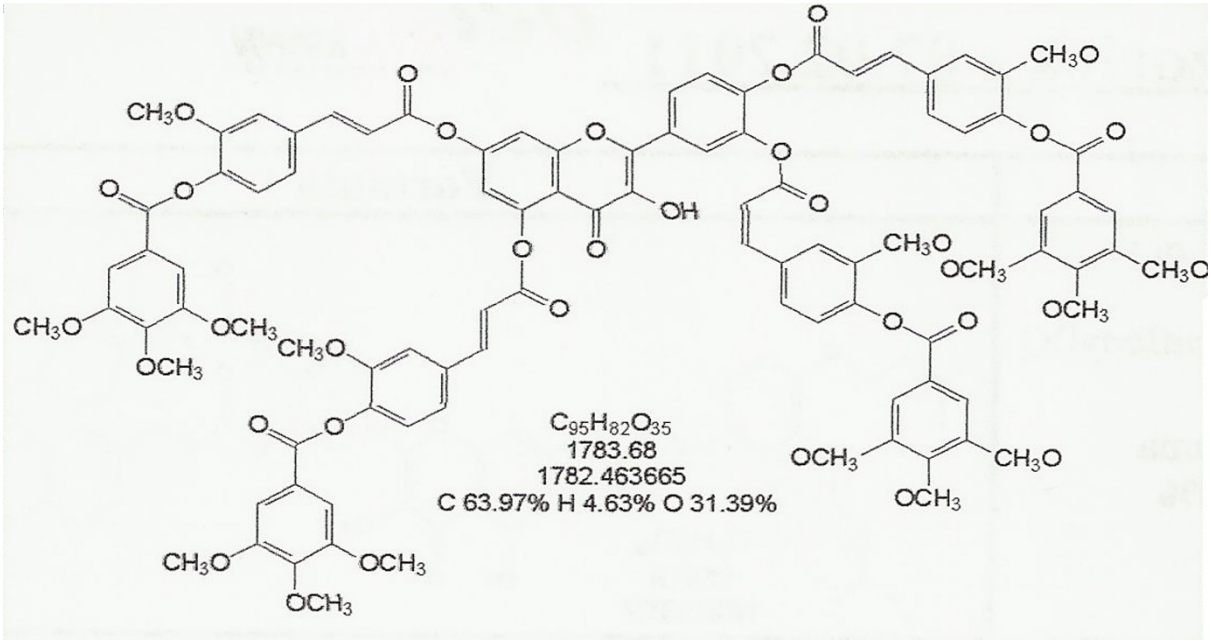
Trypan blue uptake in situ was determined by exposure of the cells to 0.1% trypan blue in phosphate buffered solution (PBS, pH 7.4). The cells were diluted with 0.1 % trypan blue (20x) and then transferred to hemocytometer and counted according to the following formula: Number of cells = mean (4/5 squares) x dilution (20x) x 10.000.

### Cytotoxicity assay

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma Aldrich) reduction assay was used as an indicator of cell damage and performed as previously described by Janjic & Wollheim (1992). INS-1E cells were seeded in 96-microwell plate at a density of $5x10^4$ cells per well. The cells were pre-incubated for 1-24 h with or without different concentrations of MGX (1–10 mM) and with or without two different concentrations of quercetin (Q) and its derivative tetra(trimethylgalloyl)-oxyferuloyl quercetin (TMFQ) (**Figure**

**1.**) (20 and 50 μM), 5% CO2, at 37 °C. MTT was added to the final concentration of 0.5 mg/ml. After 4 h of MTT incubation, solubilization buffer (10% SDS in 0.01 M HCl) was added and let to stand for 15-17 h to solubilize the formazan formed. The absorbance at 570 nm was recorded with a microplate reader (Infinite M200, Tecan, Switzerland).

**Figure 1.** The structure of tetra(trimethylgalloyl)oxyferuloyl quercetin prepared by Veverka *et al.,* 2012



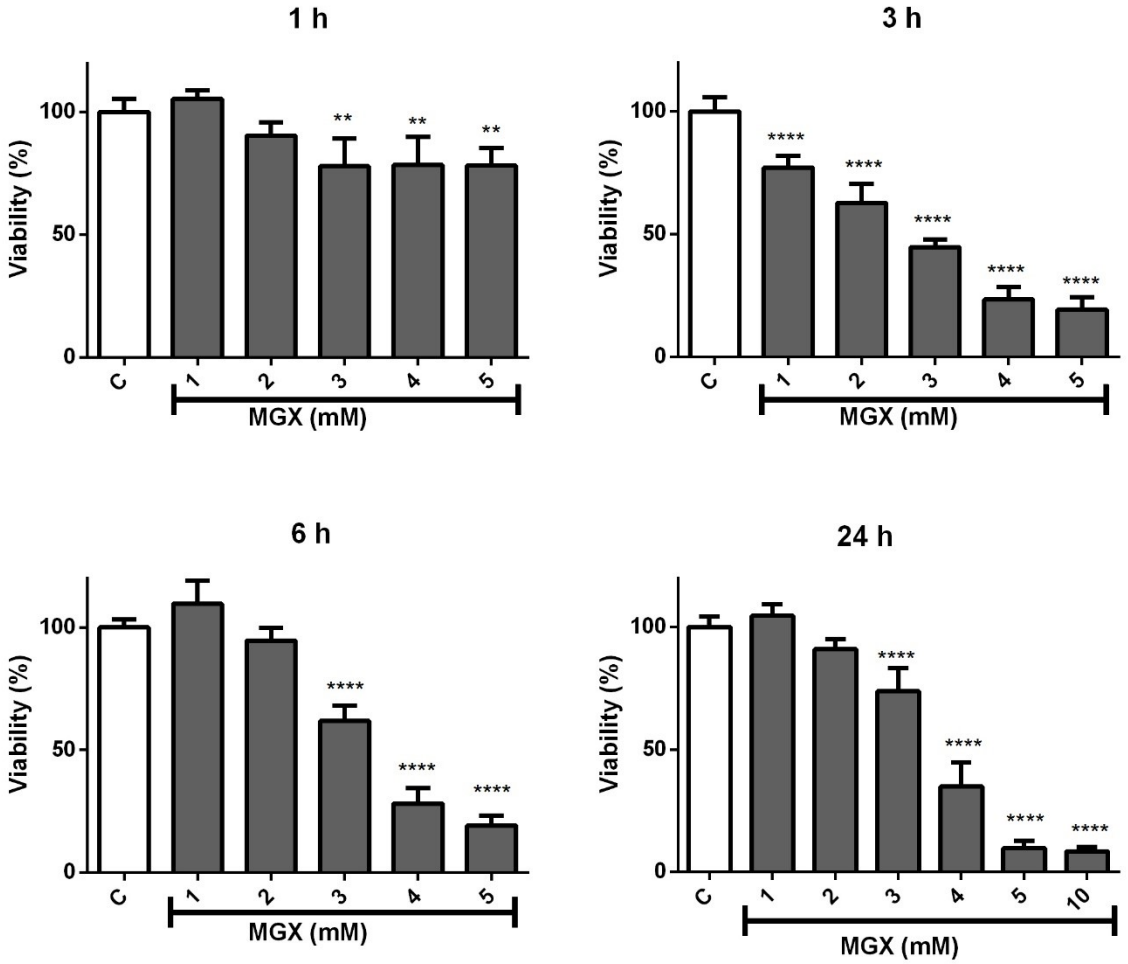

## Results and Discussion

The effect of MGX in the cultured INS-1E was determined by MTT assay. As shown in **Figure 2.**, the cells subjected to MGX (1-10 mM) showed a concentration-dependent decrease of cell viability. We also studied the effect of MGX (1-10 mM) on INS-1E cell viability at different time intervals (after 1, 3, 6 and 24 h treatment with MGX). We found that MGX treatment induced a time-dependent reduction in the cell viability.

Our results showed that MGX treatment induced a time-dependent reduction in the cell viability. In graphs 1h, 6h and 24h (**Figure 2.**) we can to research at a concentration 1mM MGX nonsignificant increase of cell viability. It could be related with effect of MGX on intracellular calcium signal pathway. Lower MGX concentrations (0–150 μM) contribute to an increased adherence of neurons on their support and an increased glia proliferation (Radu *et al.,* 2012). We may suppose that low concentrations of MGX help in intracellular calcium regulation also in pancreatic

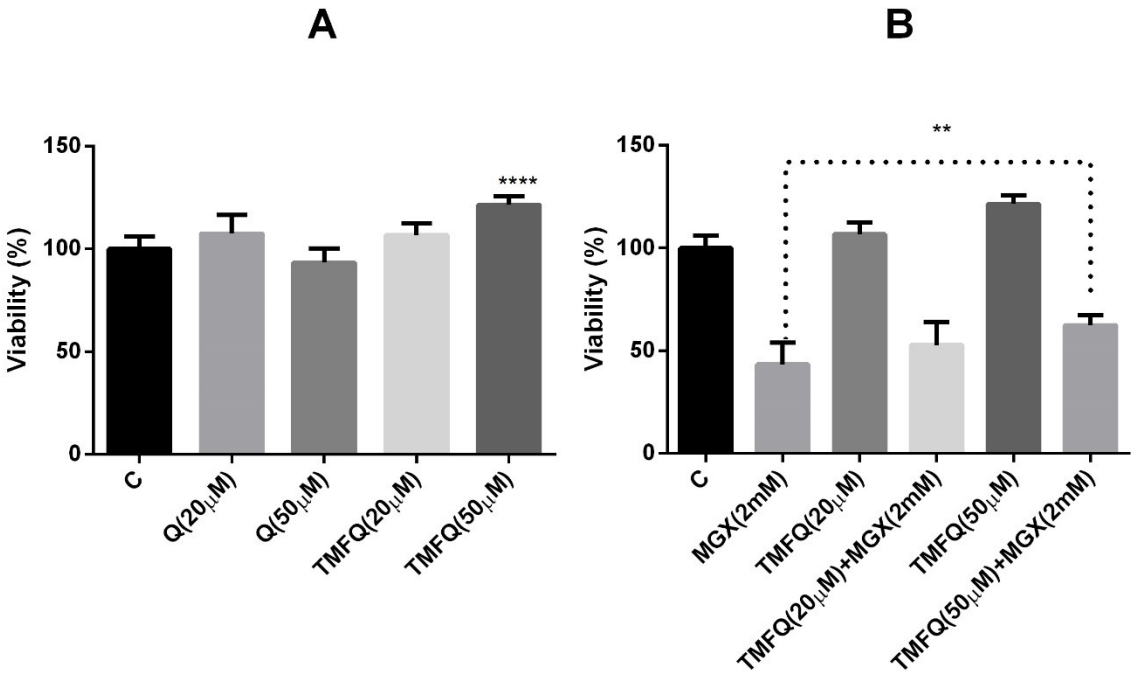
beta cells. This fact may have a positive impact on the viability. Higher concentrations of MGX evidently increase oxidative stress in cells what can lead to apoptosis and subsequent decrease in cell viability.

**Figure 2.** Time effect and concentration-dependence of methylglyoxal on INS-1E beta cell viability. Cells were treated with MGX (1-10 mM) for 1 – 24 h, and cell survival was analysed by the MTT assay. Each data point or bar represents the mean ± SD from 3 replicates. **p<0.01 and ****p<0.0001 are significant differences between the control and MGX treated samples. Statistical evaluation was performed by two-way ANOVA followed by the Bonferroni post hoc test.




Our findings concerning time effect of MGX are in accordance with those of Sheader in the time interval of 4-6 h (Sheader *et al.,* 2001). These results suggest that MGX-induced cell injury is a concentration-and time-dependent.

**Figure 3**. A - Effect of Q and its derivative TMFQ on INS-1E beta cell viability. Cells were treated for 24 h with Q or TMFQ and cell survival was analysed by the MTT assay. B- Effect of Q and its derivative TMFQ on INS-1E beta cell viability after treatment with MGX. Cells were pre-treated for 24 h with Q or TMFQ and subsequently injured by MGX (2 mM) for 3 h, and cell survival was analysed by the MTT assay. Each data point or bar represents the mean ± SD from 4 replicates. **A** ****p<0.0001 are significant differences between the control and TMFQ treated samples. **B** **p<0.01 are significant differences between the MGX treated samples and TMFQ+MGX treated samples. Statistical evaluation was performed by two-way ANOVA followed by the Bonferroni post hoc test.




Consequently, we evaluated the effect of Q and TMFQ on cell viability. Q (20 and 50 μM) was found to have no significant effect on beta cell viability, while TMFQ (50 μM) caused significant increase in beta cell viability (**Figure 3A**). We screened a series of quercetin derivatives with potential to protect the INS-1E beta cells from MGX-induced injury. Of the compounds tested, only the derivative TMFQ (50 μM) was shown to protect beta cell viability decrease from MGX-mediated impairment (**Figure 3B**). We suppose that the protective effect of TMFQ may be attributed to the presence of ferulic and gallic acid. These phenolic acids are linked to quercetin molecule via ester bonds and are known to exhibits a wide variety of biological activities, such as antioxidant, anti-inflammatory, metal chelation, modulation of enzyme activity,

activation of transcriptional factors, gene expression, signal transduction, etc. (Kumar and Pruthi, 2015). Due to the phenolic nucleus and extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical which accounts for its potent antioxidant potential. UV absorption by ferulic acid catalyses stable phenoxy radical formation and thereby potentiates its ability to terminate free radical chain reactions (Graf, 1992). This information suggests that conjugation of ferulic and gallic acid with quercetin may be responsible for the protective effect of TMFQ on INS-1E beta cell viability loss induced by MGX.

## Conclusion

To conclude, we showed the effect of MGX and TMFQ derivative on pancreatic INS-1E beta cells line. We found that TMFQ derivative significantly protected INS-1E cells from MGX-mediated viability loss. A follow-up study will be focused on the effect of TMFQ on insulin secretion, oxidative stress, apoptosis, and intracellular calcium level in INS-1E pancreatic beta cells after treatment by MGX.

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## References

[1] E. Graf , *Free Radical Biology and Medicine*, **1992**, 13, 4, 435-448.

[2] N. Kumar, V. Pruthi, *3 Biotech.*, ***2015***, 5, 4, 541–551.

[3] X. Li, T. Zheng, S. Sang, L. Lv, *J. Agric. Food Chem.*, **2014**, 62, 50, 12152–12158.

[4] B. M. Radu, D. I. Dumitrescu, C. C. Mustacuisu, M. Radu, *Cellular and Molecular Neurobiology*, **2012**, 32, 6, 1047-1057.

[5] R. P. Robertson, J. S. Harmon, *Free Radical Biology and Medicine*, **2006**, 41, 2, 177-184.

[6] E. A. Sheader, R. S. P. Benson, L. Best, *Biochemical Pharmacology*, **2001**, 61, 11, 1381-1386.

[7] R. Testa, A. R. Bonfigli, S. Genovese, V. D. Nigris, A. Ceriello, *Nutrients*, **2016**, 8, 5, 310.

[8] M. Veverka, J. Gallovic, E. Svajdlenka, E. Veverkova, N. Prónayova, I. Milackova, M. Stefek, *Chem. Pap.*, **2013**, 67, 76-83.

# Nanoparticles and nanocapsules with upconverting properties in the diagnosis of malignancies

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Cancer is one of the most common diseases in the world today. The severity of this disease is exacerbated by the unavailability of drugs and its high heterogeneity. The survival of patients, the achievement of the required clinical results of treatment and their quality of life significantly affect the time of detection of the disease. Nanotechnologies and a phenomenon called up-conversion have also found application in early detection methods.

In this work, we will explain why nanoparticles are suitable for tumour detection, as well as describe the differences between traditional fluorescence-based imaging methods compared to the use of nanocapsule technology, while highlighting its advantages and disadvantages. We will focus on the principles of diagnostics of various types of tumour cells using the above-mentioned nanocapsule technology. We also explain why up-conversion is an important phenomenon in tumour cell diagnostic technologies.

Cancer is ranked as one of the most serious civilisation diseases, since the large number of patients die from this disease every year. In 2017, there were 24,5 million incident cancer cases worldwide and 9,6 million cancer deaths [16]. The appropriate treatment for some types and stages of cancer are not clarified yet, despite modern medical technologies and procedures. The problem of its early detection is also related to the treatment possibilities, which significantly affects the survival of patients and the achievement of the desired clinical results. An early identification of cancer is key to achieving an effective treatment and improving the patient quality of life. For this purpose, new treatments are investigated in the clinical research using nanoparticles with up-conversion properties [1]. The main reason for the use of nanoparticles in medicine is the ability of enhanced permeability and retention (EPR) of nanoparticles at the tumour site.

The surface of the nanoparticles may be designed to increases the selectivity of the nanoparticles against specific tumour cells. Thanks to EPR, early imaging and detection of the tumour with relatively low spatial and temporal resolution is possible also using radiolabelled lipid vesicles for positron emission tomography (PET) [5]. Traditional fluorescence-based imaging techniques exhibit problems with low tissue penetration in the ultraviolet and visible spectral regions [14,4]. A further problem with imaging techniques is background autofluorescence, due to the use of high-power radiation, which can be also harmful and damage cells [3]. Biological imaging techniques based on the use of up-conversion (UC) phenomena can overcome these problems, since the excitation of functionalized nanoparticles occurs at lower frequencies while maintaining the emitting radiation at higher frequencies.

Several studies have been performed [2] focused as well on the technique of fluids encapsulation containing chromophores in micelles, dendrimers, and polymer shell structures. However, despite the appropriate efficacy of triplet-triplet annihilation up-conversion (TTA-UC), problems arose with structural stiffness, poor stability in required pH range, temperature fluctuations, and size distributions [13, 11].

Kwon *et al.* [8], however, developed 2 types of multifunctional nanocapsules based on a layer of silica approximately 21 nm thick, which are synthesized to encapsulate two different TTA-UC chromophore pairs. The advantage of these nanocapsules was also reflected in the increased mobility of chromophores compared to organic and inorganic nanoparticles with a solid matrix [13,12]. Each nanocapsule emits a different colour, blue (470nm) or green (505nm) after excitation with red light (635nm), depending on its composition. Using this encapsulation technique, it is possible to form capsules with different contents of up-conversion chromophores resulting in different image colours. Despite the low excitation energy, a significant increase in emission maxima and up-conversion were observed for the nanocapsules. As a result, there was no radiation damage to the cells or autofluorescence of the samples, which was a problem with conventional fluorescence imaging techniques. The synthesis strategy involves the use of oleic acid, serving as the chromophore solvent, as a micelle-forming surfactant, as well as an oxygen scavenger in TTA-UP. These nanocapsules were further conjugated to either antibodies or peptides to selectively target breast or colon cancer cells. The surface of the nanocapsules was modified by adding an amino group ($-NH_2$) for better covalent and stable binding of specific bioassays on their surface. These nanocapsules are also biocompatible due to their low cellular toxicity. Experimental results using these nanocapsules in both in vitro and in vivo systems detected cancer-

specific cells and colour-coded them in single-wavelength excitation according to the tumour type. A much greater accumulation of nanocapsules at tumour target sites due to EPR was also observed compared to fluorescence methods [8,7]. Using different pairs of chromophores for different tumour-specific colour combinations, this nanocapsule technology can also be useful in diagnosis a wide range cancer types, thanks to effective differentiation in a heterogeneous tumour microenvironment. If a suitable drug is bound together within bioassays to the surface of the tumour cells, these could be used for the treatment itself in combination with nanocapsules [9,10].

However, due to the great heterogeneity of the manifestations of cancer, which are different in patients with the same type of cancer, their identification is a difficult task. According to some studies, nanoparticles and their EPR effect are not effective in all types of tumours, as there were reported cases, where there has been no effective EPR response [15]. The effect of EPR is currently being investigated using modern imaging techniques [6]. We believe, this topic also contains high potential for further development and possibilities of future applications in science and technology, that is highly required due to the severity of the disease.

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## References

1. B. Pelaz, C. Alexiou, R. A. Alvarez-Puebla, F. Alves, A. M. Andrews, S. Ashraf, L. P. Balogh, L. Ballerini, A. Bestetti, C. Brendel, S. Bosi, M. Carril, W. C. W. Chan, C. Chen, X. Chen, X. Chen, Z. Cheng, D. Cui, J. Du, C. Dullin, *ACS Nano*, **2017**, 11, 2313−2381.

2. C. Wohnhaas, V. Mailänder, M. Dröge, M. A. Filatov, D. Brusko, Y. Avlasevich, S. Baluschev, T. Miteva, K. Landfester, A. Turshatov., *Macromol. Biosci*., **2013**, 13, 1422-1430.

3. H. Ronald; C. Marco; S. Karen. *Science and Technology of Advanced Materials*, **2019**, 20 ,1, 497-510.

4. J. S. Soenen, W. J. Parak, J. Rejman, B. Manshian., *Chem. Rev*., **2015**, 115, 2109−2135.

5. K. J. Harrington, S. Mohammadtaghi, P. S. Uster, D. Glass, A. M. Peters, R. G. Vile, J. S. Stewart., *Clin. Cancer Res*., **2001**, 7, 243−254.

6. M. Björnmalm, K. J. Thurecht, M. Michael, A. M. Scott, F. Caruso., *ACS Nano*, **2017**, 11, 9594-9613.

7. M. R. Junttila, F. J de Sauvage., *Nature*, **2013**, 501, 346-354.

8. O. S. Kwon, H. S. Song, J. Conde, H. I. Kim, N. Artzi, J. H. Kim., *ACS Nano*, **2016**, 10, 1, 1512-1521.

9. O. S. Kwon, S. H. Lee, S. J. Park, J. H. An, H. S. Song, T. Kim, J. H. Oh, J. Bae, H. Yoon, T. H. Park., *Adv. Mater*., **2013**, 25, 4177-4185.

10. O. S. Kwon, S. R. Ahn, S. J. Park, H. S. Song, H. S. Lee, J. S. Lee., *ACS Nano*, **2012**, 6, 5549-5558.

11. Q. Liu, B. Yin, T. Yang, Y. Yang, Z. Shen, P. Yao, F. Li., *J. Am. Chem. Soc*., **2013**, 135, 5029−5037.

12. Q. Liu, T. Yang, W. Feng, F. Li., *J. Am. Chem. Soc*., **2012**, 134, 5390−5397.

13. Y. C. Simon, C. Weder., *J. Mater. Chem*., **2012**, 22, 20817−20830.

14. Y. Chen, P. Xu, M. Wu, Q. Meng, H. Chen, Z. Shu, J. Wang, L. Zhang, Y. Li, J. Shi., *Adv. Mater*., **2014**, 26, 4294−4301.

15. A. Z. Natfji, D. Ravishankar, H. M I. Osborn, F. Greco., *Journal of Pharmaceutical Sciences*, **2017**, 106, 11, 3179-3187.

16. C. Fitzmaurice, T. F. Akinyemiju, F. H. A. Lami., *JAMA Oncology*, **2018**, 4, 1553-1568.

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