

Thyroid-cell derived EVs harbour surface thyrotropin-receptor and intravesicular thyroglobulin offering a novel approach for detecting thyroid cancer recurrence

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Background: Thyroid cancer (TC) poses diagnostic challenges in differentiating benign from malignant tumors and detecting recurrence, which occurs in 21–27% of patients. Analysis of serum thyroglobulin (Tg) can detect recurrence early, but the marker is not applicable in patients harboring Tg antibodies (TgAb) due to TgAb interference in standard Tg detection tests. Extracellular vesicles (EVs) present a promising target for non-invasive diagnostics as they are enriched in transmembrane proteins such as tetraspanins and tissue-specific markers. We investigated whether thyroid-cell derived EVs retain tissue specific surface characteristics (thyrotropin receptor – TSH-R) and intravesicular cargo (Tg) that can enable their use in recurrent TC detection. **Materials and Methods:** EVs were extracted via differential ultracentrifugation (dUC) from FBS-free cell culture media of normal thyroid (Nthy-Ori 3-1) and cancer cell lines (TPC-1 and OCUT2) and patient plasma (benign, malignant, recurrent). EVs number, diameter and zeta potential were assessed using Nanotracking Analysis (NTA), and their presence was confirmed by Transmission electron microscopy (TEM). Tetraspanin CD63 – an EV marker, TSH-R, and Tg - thyroid specific protein markers were detected via Dot Blot and Western Blot. **Results:** NTA detected 130–160 nm particles, in all samples implying that EV pellets were enriched with small EVs with TEM analysis confirming that conclusion. Zeta potential differed between EVs from Nthy-Ori 3-1 cell line and TPC-1-derived EVs. CD63 was present in EVs from all cell lines and patient plasma. The expression of TSH-R was higher in Nthy-Ori 3-1 cell lysates, compared to TPC-1 and OCUT2, corresponding to their differentiation state. Consistent with this, TPC-1 EVs were positive while OCUT2 EVs were negative of TSH-R, however Nthy-Ori 3-1 EVs were deprived of TSH-R. Tg was confirmed in cell lysates and EVs from Nthy-Ori 3-1 and TPC-1 EVs but absent in OCUT2 cells and EVs. EVs from plasma of patients with thyroid tumors as well as recurrence were positive for both TSH-R and Tg. The highest Tg signal was observed in a malignant sample with larger tumor size. **Conclusion:** This pilot study highlights the potential of using CD63 and TSH-R proteins for isolating thyroid-specific EVs. The presence of thyroid-specific TSH-R and intravesicular Tg in all patient samples paves the way for studies of larger sample cohorts to address early recurrence detection.

Key words: Cell line, Extracellular vesicles, Tetraspanin, Thyroid cancer, Thyroglobulin, Thyrotropin receptor

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Oncology Insights is an annual oncological open-access peer-reviewed journal that publishes new research from different areas of oncology. It strives to provide a platform for the exchange of cutting-edge research and knowledge in the field of oncology. This journal aims to advance the understanding, prevention, diagnosis, and treatment through the dissemination of high-quality scientific discoveries.

The journal applies a fair and accurate peer review process, employing double-blind review methodologies. Acceptance of manuscripts is based on their scientific merit, originality, clarity, and contribution to the field.

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Oncology Insights welcomes various types of contributions, including original research articles, review articles, case reports, case studies, clinical trials, registered reports, comments, brief communications, editorials, letters to the editor, perspectives, and conference papers from a wide range of disciplines related to cancer research.

Through encouraging interdisciplinary collaborations, the journal welcomes contributions that integrate oncology with related fields such as immunology, genetics, biochemistry, radiology, and other relevant disciplines. The journal places a special emphasis on publishing research that highlights emerging trends, novel technologies, and innovative approaches in cancer research and clinical practice.

Oncology Insights is intended for a diverse readership, including oncologists, researchers, clinicians, nurses, allied healthcare professionals, patients, patient advocates, policymakers, and all stakeholders involved in the prevention, diagnosis, and treatment of cancer. It adopts a global perspective, encompassing research from diverse regions addressing oncological challenges that may vary across different populations.

The journal is committed to upholding the highest ethical standards in research and publication provided by established international guidelines.

Periodically, Oncology Insights may publish special issues focusing on specific topics to highlight particular areas of interest or emerging needs.

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Dear colleagues,

The third volume of *Oncology Insights*, the Official Journal of the Serbian Association of Cancer Research (Srpsko društvo istraživača raka, SDIR) nicely depicts the advancements in oncology today, with a glimpse of efforts invested into broadening the scope of our research. We insist on excellence and integrity, bringing innovative approaches into our community with an open view of the specific needs of our patients.

The third volume of *Oncology Insights* features Original Research articles, Review Future Horizons articles, and Personal Points of View from researchers and patient advocates highlighting publishing integrity and advocating for wider access to oncological care.

Oncology Insights also serves as a venue for new original data from the first Regional Congress of EACR-affiliated Serbian, Croatian and Turkish societies SDIR, HDIR, and MOKAD (SHM1RC) held in Belgrade, Serbia, from October 8 to 10, 2025. The congress addressed key topics in cancer research and enhanced collaboration and the exchange of new ideas within the EACR national communities.

Lectures and short abstracts from the fields of Combination anti-cancer therapies, Clinical oncology, Molecular diagnostics in oncology, Stem cells and cancer, MDR in cancer treatment, Liquid biopsy, Cancer epidemiology, (Epi)genetics, Cancer biomarkers, New cancer therapies, Signaling pathways, Anticancer drug development, Personalized anticancer treatment, Translational oncology and Cancer radiobiology among others, are published as official Congress material.

Oncology Insights offers a platform aimed at challenging our comfort zone, discussing topics which are sometimes avoided in the everyday struggle to publish articles and prepare new projects. We invite researchers, physicians, nurses, laboratory technicians, as well as patients, survivors, caregivers, and patient advocates to offer their valuable expert insights to our journal on topics you consider important. Offering your tailored point of view stimulates the progress of oncology by collaborative efforts. We invite you to join us in shaping the future by providing evidence-based, unbiased multidisciplinary content for our future volumes, thus inspiring new lines of interest for our readers.

During these challenging times, we invite you to strive for personal and collective scientific integrity and hold on to your knowledge as your superpower. Please enjoy exploring the third volume of our journal - we hope it brings you numerous moments of intellectual freedom.

With kind regards,



Milena Čavić, SDIR President

Editor-in-Chief

Oncology Insights

Official Journal of the Serbian Association for Cancer Research





The third number of Oncology Insights includes
PROCEEDINGS BOOK of
The 1st Regional SDIR-HDIR-MOKAD Congress



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Dear Colleagues,

The organization of the 1st Regional Congress of the three national societies is now complete. It is an understatement to say that this has been an extremely demanding task, requiring the involvement of all of us over the past few months. We believe we have fulfilled our responsibilities well.

We set ourselves two important goals. The first was to create a welcoming atmosphere for every participant, allow them to expand their knowledge, present their results, establish new collaborations, and develop new scientific ideas. The second goal was to provide ample free time for participants to enjoy our beautiful city, which is especially inspiring this autumn.

We hope you will make the most of the time ahead. We also want you to take wonderful memories from Belgrade, share them with us, and inspire us to continue pursuing such initiatives. Scientific inquiry knows no boundaries and should flourish for the benefit of all, especially for those who come after us.

Sincerely,

on behalf of the Organizing Committee



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