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An electronic version of the doctoral thesis is available upon request

Breaking barriers in cancer research: the promise of a non-taxoid microtubule stabilizer and three-dimensional spheroid models

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Summary

Both taxanes and spheroids revolutionized the cancer research field. Taxanes, such as paclitaxel (PTX), were discovered during the 1960s but remain essential in the treatment of diverse cancer types. These agents target microtubules, hollow cylindrical structures involved in cell division and other cellular activities. However, clinical effectiveness of PTX therapy is hampered by side effects, especially dose-limiting peripheral neurotoxicity, and the development of PTX resistance. Accordingly, there is a pressing need for safer and effective alternatives. Crucial to drug development is the availability of preclinical cell culture models that mimic the *in vivo* situation as closely as possible and could lower the high attrition rates accompanied with drug development. One such cell culture model are spheroids: spherical, multicellular aggregates characterized by a diffusive gradient of oxygen, nutrients and catabolites. Hence recapitulating the 3D structure of avascular tumors. Spheroids are cost-effective biomimetic *in vitro* models and allow medium to high-throughput drug screening. Since recently, spheroids models can be complemented with patient-derived tumor fragments (PDTFs) that preserve the tumor heterogeneity and a near-native tumor microenvironment.

First, a new non taxoid microtubule stabilizing agent was introduced: Pelophen B (PPH). PPH is a chemical simplified analogue of peloruside A (PLA), which is originally derived from the marine sponge *Mycale hentscheli*. PLA as well as PPH bind a non-taxoid binding site on β-tubulin. We confirmed that PPH is a microtubule stabilizing agent inducing a G2/M arrest. PPH reduced metabolic activity in a diverse set of monolayer cultured cancer cells, spheroids and xenograft and patient-derived tumor fragments. Interestingly, we demonstrated that PPH remained active in PTX-resistant cells and patient-derived tumor fragments from a patient who underwent a combination of PTX and carboplatinum therapy.

Next, we introduced the Spheroid Light Microscopy Image Atlas (SLiMIA). Spheroids are abundantly used in cancer research, but there is a lack of morphometric characterization of spheroids, partly because of the lack of objective, high-throughput, automated segmentation and analysis software.

To assist the development of these algorithms, we constructed an open-access database of almost 8,000 light microscopy images covering 47 cell lines, 8 culture media, 4 spheroid formation methods, multiple seeding densities and various imaging time points. Besides training of image segmentation models, this dataset can guide spheroid researchers and aid in economization of research resources.

In conclusion, the introduction of PPH as a new microtubule stabilizing agent and the open-access SLiMIA dataset have the potential to revolutionize cancer research and cancer therapy once more.

Samenvatting

Zowel taxanen als sferoïden brachten een grote revolutie in kankeronderzoek teweeg. Taxanen zoals paclitaxel (PTX), werden reeds in de jaren '60 ontdekt maar zijn nog steeds onmisbaar voor de behandeling van kanker. Taxanen stabiliseren microtubuli. Dit zijn holle cilindrische structuren die essentieel zijn voor cellen. Behandeling met taxanen gaat helaas gepaard met bijwerkingen, vooral dosisbeperkende perifere neurotoxiciteit en de ontwikkeling van resistentie. Bijgevolg is er nood aan veiliger maar effectieve alternatieve chemotherapieën. Cruciaal voor de ontwikkeling van nieuwe geneesmiddelen is de beschikbaarheid van relevante celcultuurmodellen zoals sferoïden en tumorfragmenten.

In dit onderzoek werd eerst een nieuwe microtubuli-stabiliserende stof geïntroduceerd: Pelophen B (PPH). PPH bindt op een verschillede bindingsplaats dan PTX en vertoont anti-kanker effecten in verschillende preklinische celcultuurmodellen. Bovendien blijft PPH effectief in cellen die resistent aan taxanen. Vervolgens introduceerden we de 'Spheroid Light Microscopy Image Atlas' (SLiMIA). Dit is een vrij toegankelijke database met bijna 8000 lichtmicroscopische beelden van 47 cellijnen, 8 cultuurmedia, 4 sferoïden-vormingsmethoden, verschillende densiteiten en tijdstippen. Deze dataset kan bijdragen tot een reductie van onderzoekskosten. Daarnaast kan SLiMIA gebruikt worden voor het trainen en valideren van software die betere karakterisering van sferoïden toelaat

Tot slot, de introductie van PPH als een nieuw microtubuli-stabiliserend middel en de vrij toegankelijke SLiMIA dataset kunnen mogelijks opnieuw een revolutie teweegbrengen in kankeronderzoek en kankertherapie.

Curriculum vitae

Education	Ghent University
2017-2025	PhD in Health Sciences
2015-2017	Administrative and Technical Personnel
2013 – 2015	Master of Science in Biomedical Sciences
2009 – 2013	Bachelor of Science in Biomedical Sciences
Experience	Ghent University
2017 – 2025	Phd candidate
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Publications

Vermeulen S, Ernst S, Blondeel E et al.., Pelophen B is a non-taxoid binding microtubule-stabilizing agent with promising preclinical anticancer properties. Scientific Reports. 2024, 14(1):30188.

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Additional publications can be viewed on
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