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Preclinical evaluation of target therapeutic strategies for high-risk neuroblastoma

"A wife who loses a husband is called a widow. A husband who loses a wife is called a widower. A child who loses his parents is called an orphan. There is no word for a parent who loses a child. That's how awful the loss is."

from *An Orphan's Tale*

by Jay Neugeboren.

To all neuroblastoma patients and their families

Carolina Nunes, Eng.

Supervisor:
Prof. Dr. Frank Speleman

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PhD defense to obtain the degree of 'Doctor in Health Science'

20-12-2022

Summary

The past decades extensive genomic characterization of neuroblastoma allowed to gain insights into the genetic and clinical heterogeneity of this deadly pediatric cancer and offered tools for more refined therapeutic stratification. Neuroblastomas are characterized by typical patterns of highly recurrent DNA copy number alterations and structural variants. The overall mutational burden is low with activating *ALK* mutations in nearly 10% of cases at diagnosis and RAS/MAPK pathway mutations in relapsed cases as most prominent variants, offering opportunities for precision oncology. However, for the majority of patients options for such novel therapies are still limited while current multi-modal highly intensive therapy schemes (including GD2-immunotherapy and retinoic acid differentiation therapy) are only offering marginal gains in survival. Consequently, overall survival rates remain disappointingly low with currently still 50% chance of relapse. Moreover, long term negative effects on quality of life for survivors are very substantial. Given the current limited options for effective targeted therapies, we aimed to identify novel DNA copy number affected dependency genes for new therapeutic interventions to achieve improved survival and reduced toxicity. With this dissertation, we aimed to contribute to understand the role of the *RRM2* gene encoding a subunit of the ribonucleotide reductase (RNR) enzyme which is essential for dNTP production. We uncovered a role for *RRM2* as replicative sensor and regulator of replicative stress induced ATR-CHK1 signaling and designed a potent novel drugging combination with the RRM2 inhibitor triapine (3AP) and CHK1 inhibitor prexasertib.

Second, my experience in drug testing and data analyses also facilitated the study of the impact at molecular level of translation inhibition in neuroblastoma by CR31B, a bio-orally available silvestrol analogue. Last, during my research in drug testing, drug, drug combination and/or genetic or chemical-genetic perturbagen we noticed that the generated results were large and complex data sets, in which their analyses were time consuming. Additionally, we noticed that the scientific community lacked a tool that were able to process *in vitro* screening experiments that were assessed over time. Thus, *in vitro* screenings experiments, such as drug, drug combinations, genetic or chemical-genetic perturbagen result in large and complex data sets that require time consuming analysis. To accelerate and facilitate data processing and analysis, we developed the HTSplotter software tool to automatically process and analyze these types of experiments.

Publications in this thesis

RRM2 enhances MYCN-driven neuroblastoma formation and acts as a synergistic target with CHK1 inhibition. Carolina Nunes, Lisa Depestel, Liselot Mus, Kaylee M. Keller, et al.. Science Advances, 2022

HTSplotter: an end-to-end data processing, analysis and visualisation tool for chemical and genetic *in vitro* perturbation screening. Carolina Nunes, Jasper Anckaert, Fanny De Vloed, et.al. PLOS One. *Submitted* on 21/06/2022

TPX2 as key downstream target of the eIF4A controlled translational program in neuroblastoma. **Carolina Nunes**, Muhammad Rishfi1,2, Sarah-Lee Bekaert, Peihua Zhao, Fanny De Vloed, Fien Martens, Aline Eggermont, Frank Speleman, Anna Sablina, Kaat Durinck. *In preparation*

Publications not included in this thesis

MEIS2 is an adrenergic core regulatory transcription factor involved in early initiation of TH-MYCN driven neuroblastoma formation. Jolien De Wyn, Mark W. Zimmerman, Nina Weichert-Leahey, Carolina Nunes, et.al. Cancers, 2021

TBX2 is a neuroblastoma core regulatory circuitry component enhancing MYCN/FOXM1 reactivation of DREAM targets. Bieke Decaesteker, Geertrui Denecker, Christophe Van Neste, Emmy Dolman, Wouter Van Loocke, Moritz Gartlgruber, **Carolina Nunes**, et al. Nature Comunications, 2018