CHEMORESISTANCE PATHWAYS IN GROUP 3 MEDULLOBLASTOMA

Scientific Report Fondation FORCE

Introduction

Chemotherapy has a key role in the treatment of medulloblastoma, the most common malignant brain tumor in children and adolescents. Adjuvant chemotherapy is used across all pediatric treatment protocols, either in combination with radiotherapy in children over 3 - 5 years of age, or in intensified radiation-sparing regimens in younger children. Not only are these regimens associated with significant toxicity, tumors often acquire resistance to chemotherapy and relapse despite high dose treatment. Chemoresistance is thus a major cause of treatment failure and mortality, particularly in young children with high-risk group 3 medulloblastoma (G3 MB).

To unravel the mechanisms driving chemoresistance and relapse in G3 MB tumors, we study their evolution during treatment and characterize the pathways that promote resistance to conventional chemotherapy. Ongoing and future experiments will further test the effect of targeting these pathways to improve response to chemotherapy and prevent relapse.

Overview

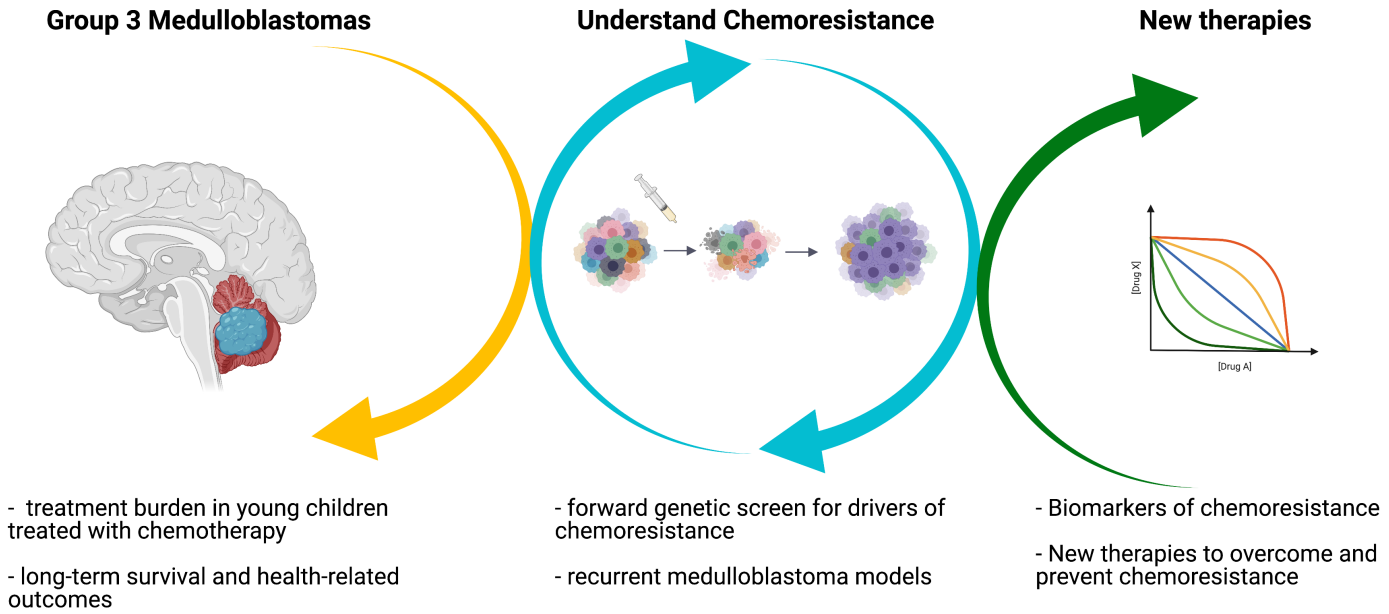


Figure 1. Integration of clinical and molecular data to understand chemoresistance in group 3 Medulloblastoma (created with BioRender.com)

Results

Outcome and treatment burden in young children (< 5 years) diagnosed with Medulloblastoma

(Note: Given the impact of the COVID19 Pandemia on experimental cancer research — which led to temporary lab closures, access restrictions and cancelation of experiments — the initial in vivo validation of candidate genes described on aim 2 is still pending. In the meantime, we added this new clinical research aim to our project).

Chemotherapy is the main postsurgical treatment option in young children with medulloblastoma. The literature on long-term outcome of patients treated at a very young age (< 5 years) for central nervous system (CNS) tumors is scarce. We sought to gain insight into the survival, health-related and employment outcomes of these patients. We retrospectively identified 164 children diagnosed with a CNS tumor under 5 years of age between 1990 and 2019 at the University Children's Hospital Zurich.

Of the 128 patients included in the final analysis, 10.2% had been diagnosed with a medulloblastoma with a 5-year PFS and OS inferior to the PFS and OS of the whole cohort (Figure 2 A, B). In addition to tumor-related death, 1 medulloblastoma patient died with a secondary malignancy. Among the patients of the whole cohort that survived and were followed for more than 5 years, 60% had neurological impairment, 32.5% neuroendocrine sequelae and18.2% with hearing impairment, and 57.1% with any visual impairment at last follow-up. In summary, the treatment burden in this very vulnerable population is high.

In vivo forward genetic screen for drivers of chemoresistance in Group 3 Medulloblastoma cells

To experimentally address the causes of treatment failure in the high-risk group of patients with Group 3 Medulloblastoma, we developed and performed an in vivo forward genetic screening for drivers of chemoresistance.

We generated D425 G3 MB cells expressing an inducible Lentihop (LH) system by lentiviral transduction of sleeping beauty (SB) transposase (SB100_GFP), Tet repressor tTR-KRAB-dsRed2 and LH transposons. Following orthotopic transplantation of transduced cells into the cerebella of NRG (NOD-*Rag1*^{null} *IL2rg*^{null}) mice, doxycycline was added to the diet for 1 week to allow SB mobilization. Tumor formation occurred within 5 weeks and mice were treated with up to 3 cycles of chemotherapy with cisplatin (day 1) and cyclophosphamide (days 2-5). Treatment with chemotherapy significantly prolonged the survival when compared to control (untreated) group (Figure 2C). At endpoint, the central nervous system was assessed for the extent of tumor recurrence. In all animals, in addition to local tumor in the posterior fossa, the presence of supratentorial/frontal and/or spinal metastases was noted (Figure 2D), similar to the metastatic behaviour observed in human group 3 medulloblastoma tumors.

Candidate genes affected by common insertion sites

Deep sequencing of DNA extracted from tumors and metastatic sites was used to determine the sleeping beauty genomic common insertion sites (gCIS). We identified transposon-induced mutations that were clonally selected in the different compartments and promoted tumour growth despite therapy. Interestingly, there were mutations specific to the metastatic sites, suggesting the possibility of distinct pathways of resistance driving metastatic tumor progression (Figure 2E).

Recurrent G3 Medulloblastoma models using patient-derived xenografts (PDXs)

We generated an additional model of recurrent G3 MB using orthotopic cerebellar patient-derived xenografts (PDXs) Med 411FH (Figure 2F), Med 114FH and Med 211 FH *(data not shown)*. Medulloblastoma PDX line Med-411FH cells were stereotactically xenografted by injection into posterior fossa of immunodeficient NRG mice. Tumors were allowed to engraft as monitored by MRI. Tumour-bearing mice (n = 9) received 3 cycles of chemotherapy with cisplatin and cyclophosphamide. RNA sequencing of primary (untreated) vs recurrent tumors from Med 411FH PDX models revealed de-regulation of DNA damage response at recurrence after chemotherapy.

Manuscripts in Preparation, supported by FORCE *(participation in congresses in 2020 impaired due to COVID 19)*

"Molecular genetics of medulloblastoma", Guerreiro Stücklin A.S., Postlmayr A., Taylor M.D., Review article, Journal of Neuro-oncology, in preparation

"Long-term Outcomes of Paediatric Brain Tumor Survivors Diagnosed at a Very Young Age", Metzger S., Weiser. A, …., Grotzer M., Guerreiro Stücklin A.S.

An additional manuscript will summarize the findings of the genetic screen, once the functional validation experiments are completed.

Subsequent Awards and Honours

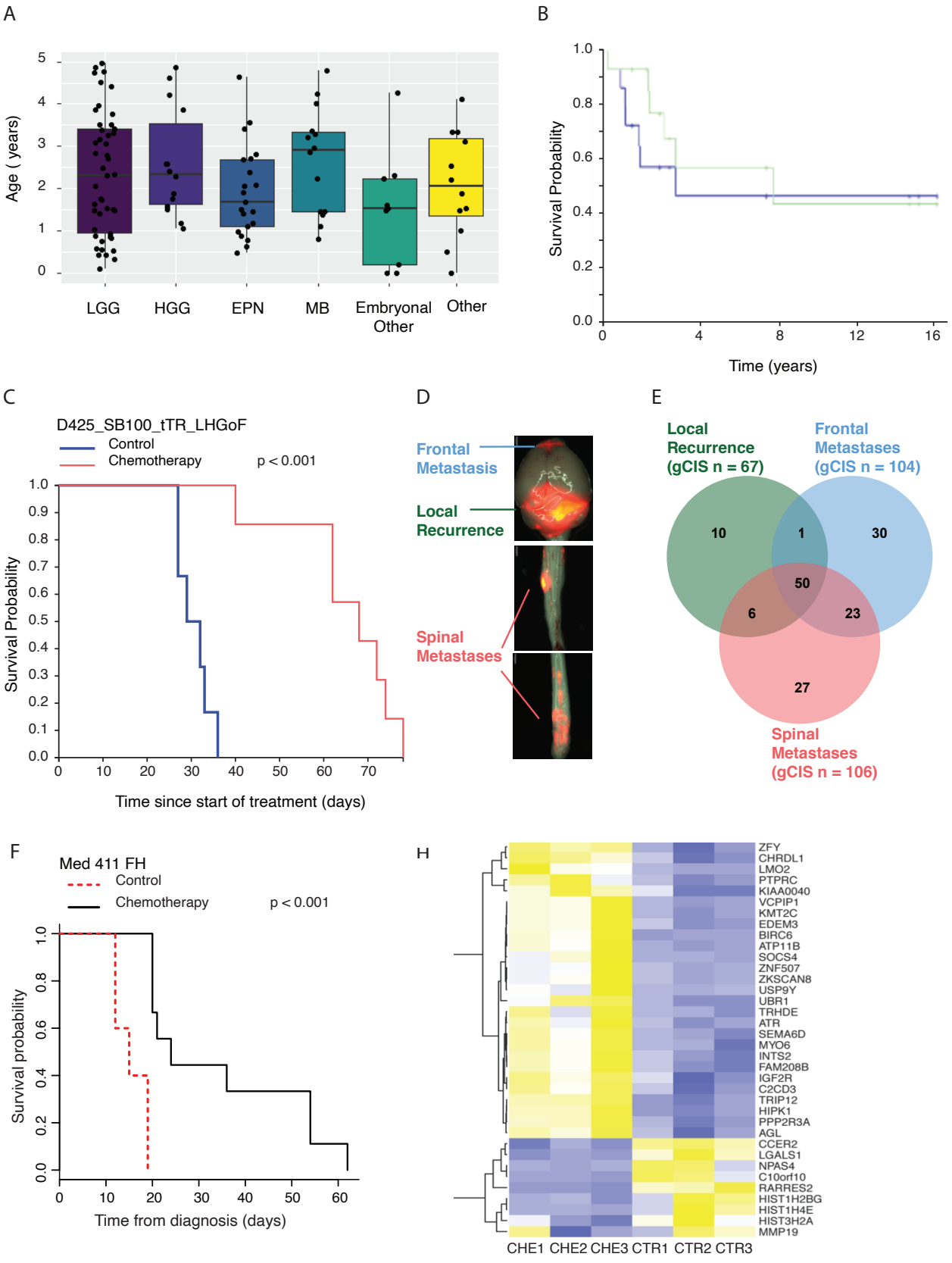
Support of Fondation FORCE was essential for launching my research group. FORCE supported my career in a vulnerable transition period as a junior PI and it was critical for the achievements that followed. In the subsequent years, I was awarded:

- Swissbridge Award 2020
- SNF Eccellenza Professorial Fellowship 2020

Future Experiments and Outlook

Our current project uses an integrated approach to 1) determine the impact and outcome of therapy in young children with medulloblastoma; 2) make use of genetic screens to understand causes of chemoresistance and treatment failure; 3) generate new concepts for therapy improvement. The molecular underpinnings of local and metastatic recurrence will be further elucidated upon functional characterization of candidate genes/pathways.

Figure 2 *(next page)*: (A) Age distribution of CNS tumors in young children; (B) Progression-free (blue lines) and Overall survival (green line) of children diagnosed with medulloblastoma under 5 years of age; (C) D425 G3 MB cells transduced with the Lentihop system. Treatment with chemotherapy improves survival as compared to controls; (D) Sites of tumor recurrence in murine CNS; (E) Venn diagram representing the overlap/divergence of gCIS in tumors after treatment (local recurrence vs supratentorial metastases vs spinal metastases); (F) Med 411FH PDXs, chemotherapy improves tumour-free survival as compared to vehicle treated cohort ; (H) RNA sequencing of primary (untreated) vs recurrent tumors from murine Ptch+/–/Math1-SB11/T2Onc (panel A) and human Med 411 FH PDX models (panel B) revealed common de-regulation of DNA damage response at recurrence after chemotherapy.


A
Age (years)
LGG
HGG
EPN
MB
Embryonal
Other
Other
B
Survival Probability
Time (years)
C
D425_SB100_tTR_LHGoF
Control
Chemotherapy
p < 0.001
Survival Probability
Time since start of treatment (days)
D
Frontal
Metastasis
Local
Recurrence
Spinal
Metastases
E
Local
Recurrence
(gCIS n = 67)
Frontal
Metastases
(gCIS n = 104)
10
1
30
50
6
23
27
Spinal
Metastases
(gCIS n = 106)
F
Med 411 FH
Control
Chemotherapy
p < 0.001
Survival probability
Time from diagnosis (days)
H
ZFY
CHRDL1
LMO2
PTPRC
KIAA0040
VCPIP1
KMT2C
EDEM3
BIRC6
ATP11B
SOCS4
ZNF507
ZKSCAN8
USP9Y
UBR1
TRHDE
ATR
SEMA6D
MYO6
INTS2
FAM208B
IGF2R
C2CD3
TRIP12
HIPK1
PPP2R3A
AGL
CCER2
LGALS1
NPAS4
C10orf10
RARRES2
HIST1H2BG
HIST1H4E
HIST3H2A
MMP19
CHE1 CHE2 CHE3 CTR1 CTR2 CTR3