Group 3 Medulloblastoma

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Medulloblastoma is the most common malignant pediatric brain tumor, which accounts for approximately 20% of all childhood brain tumors.

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1. Introduction

Heterogeneity is a main feature of cancer and prevents the development of targeted therapies in the vast majority of cases. The recent molecular characterization of several tumors has greatly contributed to overcome this issue and has provided a new interpretation of each cancer type as a set of individual tumors. Unveiling the molecular basis underlying each tumor subtype is a requisite for instructing personalized cancer treatments. Such deeply molecular analysis has been successfully carried out in Medulloblastoma (MB), the most common malignant pediatric brain tumor [1] arising from progenitor cell populations during early brain development. It has been classified into the four molecular subgroups Wingless (WNT), Sonic Hedgehog (SHH), Group 3 (G3) and Group 4 (G4), with defined features both in terms of driver genes and clinical outcomes [2][3]. The most aggressive MB subgroup is the G3, accounting for 25% of all MBs and associated with the highest rate of metastasis at diagnosis (about 45%) and the worst survival outcome at 5 years (under 60%) [4][5]. Until now, a G3 common driver pathway has not been identified, which makes this subgroup still enigmatic. However, a c-MYC signature is restricted to G3: it occurs in about 17% of patients whereas it is extremely rare in other MB subgroups, G4 being characterized by n-MYC signature [2][3][6]. In particular, MYC alteration is due to both gene amplification and aberrant expression and is strictly related to unfavorable outcomes [4][7][8][9]. Notably, it has been recently demonstrated that the concomitant overexpression of *MYC* and *OTX*, another relevant driver gene in G3, is sufficient to induce MB in vivo [10].

c-MYC (referred to as MYC hereinafter) is an essential transcriptional factor that orchestrates the gene expression programs necessary for growth, expansion and homeostasis of somatic and stem cells [11]. Together with its protein partner MAX, and other cofactors, MYC drives transcription of at least 15% of all genes [12], both protein-coding and noncoding genes [13][14][15].

While in normal cells the levels of *MYC* RNA and protein are tightly regulated, in tumor cells they are aberrantly expressed. High levels of MYC have dramatic effects due to the amplification of the ongoing gene expression programs and the activation of previously silent genes implicated in cell cycle progression, proliferation, migration and metastasis [16]. For these reasons, MYC has long been considered an ideal cancer target [17]. Interfering with MYC expression or function was regarded as potentially detrimental for normal cells for a long time. However, recently the use of small peptides as MYC inhibitors proved to be a safe and effective therapeutic strategy [18]. Being deregulated in up to 70% of human cancers [19], MYC inhibition can be in principle used against multiple types of tumor.

By contrast, the development of tailored therapies implies a deeper knowledge of tumor-specific genes. The continued efforts in refining MB classification have provided a new challenge for identifying novel, specific tumor drivers beyond the well-known protein coding genes. In this framework, a specific class of noncoding transcripts, the long noncoding RNAs (lncRNAs), are emerging as attractive players in shaping MB features [20]. Because of their assessed role as oncogenes or oncosuppressors and their high tissue- and cancer-type specificity, they perfectly meet the criteria for being promising tumor targets and biomarkers. Nevertheless, for G3 MB mainly in silico studies have been carried out to pinpoint lncRNAs potentially involved in tumor biology [21].

2. Novel MYC-Regulated Long Noncoding RNAs in Group 3

Medulloblastoma

Cancer is a very complex pathology characterized by high heterogeneity among patients and tumor types [22][23]. It is well established that both coding and non-coding mutations greatly contribute to cancer biology. In particular, the vast majority of known driver mutations affect protein-coding regions and are mainly responsible for aberrant chromatin remodeling and proliferation pathway alterations [24]. Nevertheless, a number of genetic perturbations that affect developmental pathways, such as WNT and NOTCH, are also produced by somatic mutations in non-coding regions as the *cis* regulatory (promoters) and enhancer sequences or the untranslated regions (5′ and 3′UTRs), all of which may have a strong impact on gene expression [24].

Moreover, recent studies that combined DNA- and RNA-based approaches to identify cancer-associated pathways have greatly expanded our knowledge of the multiple mechanisms underlying tumor biology. They revealed that some alterations may occur through changes in RNA, rather than DNA sequence mutations, such as overexpression [25], altered splicing [26] and gene fusion [27].

To add a further layer of complexity, genomic alterations in coding genes that control multiple targets—as chromatin regulators or transcription factors—may in turn affect noncoding genes. This is the case of lncRNAs, noncoding transcripts that are widely implicated in the regulation of gene expression programs underlying relevant biological processes, such as cell differentiation and development. They may act both in *cis* and in *trans* and, because of their modular nature, have the unique property to interact with proteins as well as with nucleic acids, both DNA and RNA, with high specificity. Notably, their ability to simultaneously establish such interactions provides them the possibility of targeting specific factors/complexes to a single location [28][29].

In line with their roles, the aberrant expression of lncRNAs may profoundly affect cellular pathways with pathological outcomes. Nowadays, they have been increasingly implicated in tumorigenesis and have been shown to contribute to each of the cancer hallmarks, from cell proliferation and survival to apoptosis, invasion and angiogenesis [30][31]. Such implication arose from the observation that lncRNA expression may be regulated by key oncogenic transcription factors such as MYC, which is involved in the majority of human tumors [19][32]. This suggested the possibility that these transcripts may play a part in the functional output of the oncogenic signal. Notably, while MYC is expressed in a variety of tumors, the lncRNAs are endowed with cell- and cancer type-specific expression which suggests that the same oncogene MYC may influence the expression of distinct sets of lncRNAs, depending on the pathological context. Therefore, while MYC is regarded as a universal target in cancer [18], the identification of MYC-responsive lncRNAs in distinct tumors may represent an alternative strategy for unveiling novel biomarkers as well as driver genes and therapeutic targets. They may be powerful biomarkers not only because many of them are uniquely expressed in distinct cancer types [33], but also because they may be easily detected in body fluids, such as urine, blood and cerebrospinal fluids, making the tumor diagnosis less invasive [34]. Their activity as driver genes is tightly dependent on their role as crucial nodes of regulatory networks. Paradigmatic is their action as microRNA sponges that may derepress gene expression in a pleiotropic manner [35][36] or as scaffolds to deliver transcriptional factors or chromatin remodeling complexes to the chromatin site [37][38].

In the era of precision oncology, researchers decided to unveil the contribution of lncRNAs to MB, the most common pediatric malignant brain tumor, mainly occurring in children under the age of ten [39]. To date, no pharmacological approaches are decisive in the treatment of this tumor, while the secondary effects of chemotherapy, craniospinal radiation or surgical interventions heavily affect the quality of life of pediatric patients, requiring the rapid development of alternative therapies. In particular, G3 MB subgroup sparked our interest for a number of reasons: (i) it is the most aggressive subgroup, being associated with the highest rate of metastasis at diagnosis (40–45%) and the worst survival outcome (under 60% at 5 years) [4][5]; (ii) no any univocal driver pathway has been underscored so far; (iii) the lncRNA landscape has never been thoroughly explored [40].

To unveil the lncRNAs engaged in G3 MB tumorigenesis, researchers exploited a feature of this subgroup, namely the high MYC level, due to both gene copy number increase and aberrant expression [3][10]. Therefore, we looked for MYC-dependent lncRNAs in a cell line, the D283 MED cells, in which *MYC* is overexpressed [41].

To this aim, researchers inhibited MYC function through the well-characterized dominant-negative OMOMYC [18][42] and analyzed the resultant impact on cell transcriptome. The advantage of using OMOMYC strategy is at least double. On the one hand, by sequestering MYC away from E-boxes on the promoter regions of target genes, OMOMYC blocks the expression of the MYC gene signature, common to tumors with high MYC expression [43]. Additionally, OMOMYC was also proposed to form transcriptionally inactive homodimers to E-boxes, resulting in inhibition of MYC target gene

expression [18]. On the other hand, OMOMYC, interfering with the binding of MYC to its partner MAX, leads to ubiquitination and proteasome-dependent degradation of the free MYC monomer [44].

This strategy allowed us to compile the first atlas of MYC-dependent lncRNAs in G3 MB [10]. Through a stringent filtering procedure, researchers selected three candidates, renamed *lncMB1*, *lncMB2* and *lncMB3*, to be tested for their involvement in G3 MB biology.

By comparing their expression profile in MYC-driven MB-derived cells and G3 primary tumors to normal cerebella, researchers hypothesized a potential oncogenic role for all of them.

By testing the ability of the lncRNAs to influence tumor cell-related features, researchers highlighted a role for *lncMB3* in evading programmed cell death. Apoptosis, as a protective mechanism devoted to the maintenance of tissue homeostasis, represents a natural barrier that should be circumvented during tumor development [45]. Accordingly, the acquired resistance towards apoptosis is a hallmark of most, if not all, types of cancer. In this context, the future discovery of *lncMB3* target genes will provide a new molecular pathway underlying G3 MB pathogenesis.

Starting from the discovery of the oncogene ZNF703 [46][47][48][49] as a *lncMB2* target gene, researchers were able to demonstrate a role for the lncRNA in promoting cell migration and invasion, processes underlying cancer metastasis [50]. However, further studies are needed to realize whether this capability is mediated by ZNF703 and/or by other regulatory circuitries.

References

1. Louis, D.N.; Perry, A.; Reifenberger, G.; von Deimling, A.; Figarella-Branger, D.; Cavenee, W.K.; Ohgaki, H.; Wiestler, O.D.; Kleihues, P.; Ellison, D.W. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: A summary. Acta Neuropathol. 2016, 131, 803–820.
2. Northcott, P.A.; Buchhalter, I.; Morrissy, S.; Hovestadt, V.; Weischenfeldt, J.; Ehrenberger, T.; Gröbner, S.; Segura-Wang, M.; Zichner, T.; Rudneva, V.; et al. The whole-genome landscape of medulloblastoma subtypes. Nat. Cell Biol. 2017, 547, 311–317.
3. Cavalli, F.M.; Remke, M.; Rampasek, L.; Peacock, J.; Shih, D.J.H.; Luu, B.; Garzia, L.; Torchia, J.; Nor, C.; Morrissy, S.; et al. Intertumoral Heterogeneity within Medulloblastoma Subgroups. Cancer Cell 2017, 31, 737–754.e6.
4. Northcott, P.A.; Shih, D.J.H.; Remke, M.; Cho, Y.-J.; Kool, M.; Hawkins, C.; Eberhart, C.G.; Dubuc, A.; Guettouche, T.; Cardentey, Y.; et al. Rapid, reliable, and reproducible molecular sub-grouping of clinical medulloblastoma samples. Acta Neuropathol. 2011, 123, 615–626.
5. Taylor, M.D.; Northcott, P.A.; Korshunov, A.; Remke, M.; Cho, Y.-J.; Clifford, S.C.; Eberhart, C.G.; Parsons, D.W.; Rutkowski, S.; Gajjar, A.; et al. Molecular subgroups of medulloblastoma: The current consensus. Acta Neuropathol. 2011, 123, 465–472.
6. Clifford, S.C.; Lusher, M.E.; Lindsey, J.C.; Langdon, J.A.; Gilbertson, R.J.; Straughton, D.; Ellison, D.W. Wnt/Wingless Pathway Activation and Chromosome 6 Loss Characterise a Distinct Molecular Sub-Group of Medulloblastomas Associated with a Favourable Prognosis. Cell Cycle 2006, 5, 2666–2670.
7. Cho, Y.-J.; Tsherniak, A.; Tamayo, P.; Santagata, S.; Ligon, A.; Greulich, H.; Berhoukim, R.; Amani, V.; Goumnerova, L.; Eberhart, C.G.; et al. Integrative Genomic Analysis of Medulloblastoma Identifies a Molecular Subgroup That Drives Poor Clinical Outcome. J. Clin. Oncol. 2011, 29, 1424–1430.
8. Kool, M.; Koster, J.; Bunt, J.; Hasselt, N.E.; Lakeman, A.; Van Sluis, P.; Troost, D.; Meeteren, N.S.-V.; Caron, H.N.; Cloos, J.; et al. Integrated Genomics Identifies Five Medulloblastoma Subtypes with Distinct Genetic Profiles, Pathway Signatures and Clinicopathological Features. PLoS ONE 2008, 3, e3088.
9. Robinson, G.; Parker, M.; Kranenburg, T.; Lu, C.; Chen, X.; Ding, L.; Phoenix, T.N.; Hedlund, E.; Wei, L.; Zhu, X.; et al. Novel mutations target distinct subgroups of medulloblastoma. Nat. Cell Biol. 2012, 488, 43–48.
10. Ballabio, C.; Anderle, M.; Gianesello, M.; Lago, C.; Miele, E.; Cardano, M.; Aiello, G.; Piazza, S.; Caron, D.; Gianno, F.; et al. Modeling medulloblastoma in vivo and with human cerebellar organoids. Nat. Commun. 2020, 11, 1–18.
11. Oster, S.K.; Ho, C.S.; Soucie, E.; Penn, L. The myc Oncogene: Omplex. Adv. Cancer Res. 2002, 84, 81–154.
12. Wang, C.; Fang, H.; Zhang, J.; Gu, Y. Targeting “undruggable” c-Myc protein by synthetic lethality. Front. Med. 2021, 1–10.
13. Van Dang, C. Enigmatic MYC Conducts an Unfolding Systems Biology Symphony. Genes Cancer 2010, 1, 526–531.

14. Dang, C.V.; O'Donnell, K.A.; Zeller, K.I.; Nguyen, T.; Osthus, R.C.; Li, F. The c-Myc target gene network. Semin. Cancer Biol. 2006, 16, 253–264.
15. Patel, J.H.; Loboda, A.P.; Showe, M.K.; Showe, L.C.; McMahon, S.B. Analysis of genomic targets reveals complex functions of MYC. Nat. Rev. Cancer 2004, 4, 562–568.
16. Smith, K.; Dalton, S. Myc transcription factors: Key regulators behind establishment and maintenance of pluripotency. Regen. Med. 2010, 5, 947–959.
17. Whitfield, J.; Beaulieu, M.-E.; Soucek, L. Strategies to Inhibit Myc and Their Clinical Applicability. Front. Cell Dev. Biol. 2017, 5, 10.
18. Massó-Vallés, D.; Soucek, L. Blocking Myc to Treat Cancer: Reflecting on Two Decades of Omomyc. Cells 2020, 9, 883.
19. Dang, C.V. MYC on the Path to Cancer. Cell 2012, 149, 22–35.
20. Laneve, P.; Caffarelli, E. The Non-coding Side of Medulloblastoma. Front. Cell Dev. Biol. 2020, 8, 275.
21. Kesherwani, V.; Shukla, M.; Coulter, D.W.; Sharp, J.G.; Joshi, S.S.; Chaturvedi, N.K. Long non-coding RNA profiling of pediatric Medulloblastoma. BMC Med Genom. 2020, 13, 1–14.
22. Vogelstein, B.; Papadopoulos, N.; Velculescu, V.; Zhou, S.; Diaz, L.; Kinzler, K.W. Cancer Genome Landscapes. Science 2013, 339, 1546–1558.
23. Dagogo-Jack, I.; Shaw, A.T. Tumour heterogeneity and resistance to cancer therapies. Nat. Rev. Clin. Oncol. 2017, 15, 81–94.
24. Reyna, M.A.; PCAWG Drivers and Functional Interpretation Working Group; Haan, D.; Paczkowska, M.; Verbeke, L.P.C.; Vazquez, M.; Kahraman, A.; Pulido-Tamayo, S.; Barenboim, J.; Wadi, L.; et al. Pathway and network analysis of more than 2500 whole cancer genomes. Nat. Commun. 2020, 11, 729.
25. Owens, M.A.; Horten, B.C.; Da Silva, M.M. HER2 Amplification Ratios by Fluorescence In Situ Hybridization and Correlation with Immunohistochemistry in a Cohort of 6556 Breast Cancer Tissues. Clin. Breast Cancer 2004, 5, 63–69.
26. Climente-González, H.; Porta-Pardo, E.; Godzik, A.; Eyras, E. The Functional Impact of Alternative Splicing in Cancer. Cell Rep. 2017, 20, 2215–2226.
27. Faderl, S.; Talpaz, M.; Estrov, Z.; O'Brien, S.; Kurzrock, R.; Kantarjian, H.M. The Biology of Chronic Myeloid Leukemia. N. Engl. J. Med. 1999, 341, 164–172.
28. Statello, L.; Guo, C.-J.; Chen, L.-L.; Huarte, M. Gene regulation by long non-coding RNAs and its biological functions. Nat. Rev. Mol. Cell Biol. 2020, 22, 96–118.
29. Kopp, F.; Mendell, J.T. Functional Classification and Experimental Dissection of Long Noncoding RNAs. Cell 2018, 172, 393–407.
30. Gutschner, T.; Diederichs, S. The hallmarks of cancer. RNA Biol. 2012, 9, 703–719.
31. Wang, J.; Zhang, X.; Chen, W.; Hu, X.; Li, J.; Liu, C. Regulatory roles of long noncoding RNAs implicated in cancer hallmarks. Int. J. Cancer 2019, 146, 906–916.
32. Hart, J.; Roberts, T.C.; Weinberg, M.; Morris, K.; Vogt, P.K. MYC regulates the non-coding transcriptome. Oncotarget 2014, 5, 12543–12554.
33. Iyer, M.K.; Niknafs, Y.S.; Malik, R.; Singhal, U.; Sahu, A.; Hosono, Y.; Barrette, T.R.; Prensner, J.; Evans, J.R.; Zhao, S.; et al. The landscape of long noncoding RNAs in the human transcriptome. Nat. Genet. 2015, 47, 199–208.
34. Schmitt, A.M.; Chang, H.Y. Long Noncoding RNAs in Cancer Pathways. Cancer Cell 2016, 29, 452–463.
35. Laneve, P.; Po, A.; Favia, A.; Legnini, I.; Alfano, V.; Rea, J.; Di Carlo, V.; Bevilacqua, V.; Miele, E.; Mastronuzzi, A.; et al. The long noncoding RNA linc-NeD125 controls the expression of medulloblastoma driver genes by microRNA sponge activity. Oncotarget 2017, 8, 31003–31015.
36. Yang, C.; Wu, D.; Gao, L.; Liu, X.; Jin, Y.; Wang, D.; Wang, T.; Li, X. Competing endogenous RNA networks in human cancer: Hypothesis, validation, and perspectives. Oncotarget 2016, 7, 13479–13490.
37. Sun, Q.; Hao, Q.; Prasanth, K.V. Nuclear Long Noncoding RNAs: Key Regulators of Gene Expression. Trends Genet. 2018, 34, 142–157.
38. Morlando, M.; Fatica, A. Alteration of Epigenetic Regulation by Long Noncoding RNAs in Cancer. Int. J. Mol. Sci. 2018, 19, 570.

39. Ostrom, Q.T.; Gittleman, H.; Truitt, G.; Boscia, A.; Kruchko, C.; Barnholtz-Sloan, J. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011–2015. Neuro-Oncology 2018, 20, iv1–iv86.
40. Laneve, P.; Rea, J.; Caffarelli, E. Long Noncoding RNAs: Emerging Players in Medulloblastoma. Front. Pediatr. 2019, 7.
41. Ivanov, D.P.; Coyle, B.; Walker, D.A.; Grabowska, A.M. In vitro models of medulloblastoma: Choosing the right tool for the job. J. Biotechnol. 2016, 236, 10–25.
42. Soucek, L.; Helmer-Citterich, M.; Sacco, A.; Jucker, R.; Cesareni, G.; Nasi, S. Design and properties of a Myc derivative that efficiently homodimerizes. Oncogene 1998, 17, 2463–2472.
43. Jung, L.A.; Gebhardt, A.; Koelmel, W.; Ade, C.P.; Walz, S.; Kuper, J.; von Eyss, B.; Letschert, S.; Redel, C.; D'Artista, L.; et al. OmoMYC blunts promoter invasion by oncogenic MYC to inhibit gene expression characteristic of MYC-dependent tumors. Oncogene 2016, 36, 1911–1924.
44. Demma, M.J.; Mapelli, C.; Sun, A.; Bodea, S.; Ruprecht, B.; Javaid, S.; Wiswell, D.; Muise, E.; Chen, S.; Zelina, J.; et al. Omomyc Reveals New Mechanisms To Inhibit the MYC Oncogene. Mol. Cell. Biol. 2019, 39.
45. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The Next Generation. Cell 2011, 144, 646–674.
46. Yang, G.; Ma, F.; Zhong, M.; Fang, L.; Peng, Y.; Xin, X.; Zhong, J.; Yuan, F.; Gu, H.; Zhu, W.; et al. ZNF703 acts as an oncogene that promotes progression in gastric cancer. Oncol. Rep. 2014, 31, 1877–1882.
47. Wang, S.; Wang, C.; Hu, Y.; Li, X.; Jin, S.; Liu, O.; Gou, R.; Zhuang, Y.; Guo, Q.; Nie, X.; et al. ZNF703 promotes tumor progression in ovarian cancer by interacting with HE4 and epigenetically regulating PEA15. J. Exp. Clin. Cancer Res. 2020, 39, 1–19.
48. Klæstad, E.; Sawicka, J.E.; Engstrøm, M.J.; Ytterhus, B.; Valla, M.; Bofin, A.M. ZNF703 gene copy number and protein expression in breast cancer; associations with proliferation, prognosis and luminal subtypes. Breast Cancer Res. Treat. 2021, 186, 65–77.
49. Guo, J.; Luo, C.; Yang, Y.; Dong, J.; Guo, Z.; Yang, J.; Lian, H.; Ye, C.; Liu, M. MiR-491-5p, as a Tumor Suppressor, Prevents Migration and Invasion of Breast Cancer by Targeting ZNF-703 to Regulate AKT/mTOR Pathway. Cancer Manag. Res. 2021, 13, 403–413.
50. Friedl, P.; Wolf, K. Plasticity of cell migration: A multiscale tuning model. J. Cell Biol. 2009, 188, 11–19.