SHORT COMMUNICATION

A new mouse model for c-Myc induced choroid plexus carcinoma

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Choroid plexus carcinomas (CPCs) are World Health Organization [WHO] grade III brain tumors predominantly found in children (1,2). Implementation of successful therapy for CPCs has been hampered by the lack of appropriate preclinical models. Here we review the *Otx2*$^{CreER/+}$; *Rosa*$^{MycT58A/MycT58A}$; *Trp*$^{53fl/fl}$ novel CPC mouse model we recently generated, and compare it to existing models (Table 1). CPCs derive from the choroid plexus (CP), a secretory epithelium of the lateral, third and fourth brain ventricles that is essential for the formation and maintenance of the brain through production of the cerebrospinal fluid (3,4). These highly malignant tumors are characterized by large chromosomal alterations, confusing the identification of the genes actually involved in tumorigenesis (5-7). CPCs have been associated to TP53 germline and somatic mutations, and more recently to c-MYC overexpression, suggesting that these genetic alterations play a crucial role in CPC tumorigenesis (8-10). Improved therapy for these cancers depends on the generation of animal models closely reproducing the genetics of human tumor susceptibility, which can then be used to study tumor biology and for preclinical testing. CPCs were initially observed in transgenic mice expressing SV40 large T antigen, which alters Trp53 and retinoblastoma (Rb) function (11-13). These mice developed various choroid plexus tumors (carcinomas but also benign papillomas) with different latency and incidence rates. However, these models did not allow spatial or temporal control of genetic alterations. More recently, refined approaches were used to incorporate conditional genetic alterations in specific tissues and/or with temporal control. In 2015, Tong et al. generated two novel mouse models of CP tumors by *in utero* electroporation of a Cre recombinase-expressing plasmid into the fourth ventricle of Trp53$^{fl:fl}$; Rb$^{fl:fl}$ or Trp53$^{fl:fl}$; Rb$^{fl:fl}$; Pten$^{fl:fl}$ embryos (14). This provoked the formation of CPCs similar to human tumors in 10% and 38% of the cases, respectively, and led to the identification of a group of three oncogenes concurrently gained in CPCs: TAF12, NFYC and RAD54L, which might favour tumoral progression by promoting aberrant DNA repair and epigenome remodelling. In 2017, Kawauchi et al. (15) used a similar approach to conditionally overexpress c-Myc and inactivate Trp53 in various embryonic subpopulations via *in utero* electroporation of Blbp$^{Cre/+}$ and *Atoh1*$^{Cre/+}$ embryos. Although these models were initially designed to get type 3 medulloblastomas (MBs), another paediatric cancer with frequent overexpression of c-Myc, CPC were frequently observed in both genetic backgrounds. This was attributed to partial expression of the Cre-genetic drivers in choroid plexus cells, in addition to cerebellar precursors from which MBs normally occur. Interestingly, although tumoral development occurred with a total penetrance in these systems, CPC and MB were never obtained in the same animal, suggesting that inhibitory mechanisms might come into play between these two types of tumors. Lately, we designed a new genetically-engineered mouse model of CPC (16). In this model, expression of a stabilised form of c-Myc (MycT58A) and ablation of Trp53 can be induced by an Otx2-driven, tamoxifen-inducible Cre recombinase (*Otx2*$^{CreER/+}$; *Rosa*$^{MycT58A/MycT58A}$; *Trp53*$^{fl/fl}$). This system enables to target the two genetic alterations most frequently observed in human CPCs directly into the choroid plexus of all brain ventricles, and at any development stage, since Otx2 is strongly expressed in all choroid plexuses from embryogenesis till adulthood (16). In contrast, electroporation is restricted temporally to specific embryonic stages (E12.5-E13.5) and spatially to choroid plexus of the fourth ventricle. Induction of these alterations in

TABLE 1

Overview of mouse models of choroid plexus carcinomas (CPCs)

Mouse genotype	Genetic alteration(s) induction method	Induction Stage	Target cells	Tumor latency	Tumor incidence	Tumor Location	Notes	Ref
Wild type	Eggs microinjected with SV40 T expression vector	E0	Whole embryo	1-5 months	64% (n=16/25)	Lateral, 3th and 4th ventricles	CPT	[10]
Wild type	Single cell embryos microinjected with modified SV40 T expression vector	E0	Whole embryo	11-44 weeks		Lateral, 3th and 4th ventricles	CPT	[9]
Trp53$^{-/-}$	Single cell embryos microinjected with modified SV40 T expression vector	E0	Whole embryo	1 month	100% (n= 4/4)	Lateral, 3th and 4th ventricles	CPT	[11]
Trp53$^{fl/fl}$; *Rb*$^{fl/fl}$	4th ventricle *in utero* eletroporation of Cre recombinase plasmid	E12,5	Electroporated cells	3-10 months	10% (n=7/68)	4th ventricle		[12]
Trp53$^{fl/fl}$; *Rb*$^{fl/fl}$; *Pten*$^{fl/fl}$	4th ventricle *in utero* eletroporation of Cre recombinase expression vector	E12,5	Electroporated cells	2-8 months	38% (n=26/69)	4th ventricle		[12]
Blbp$^{Cre/+}$	4th ventricle *in utero* electroporation of Myc and dominant negative Trp53 expression vectors	E13,5	Blbp-electroporated cells	1-2 months	43% (n=6/14)	4th ventricle	MB 57% (n=8/14)	[13]
Atoh1$^{Cre/+}$	4th ventricle *in utero* electroporation of Myc and dominant negative Trp53 expression vectors	E13,5	Atoh1-electroporated cells	1 month	67% (n=2/3)	4th ventricle	MB 33% (n=1/3)	[13]
Otx2$^{CreER/+}$; *Rosa*$^{MycT58A/MycT58A}$; *Trp53*$^{fl/fl}$	Tamoxifen injection	P1-P7	Otx2-positive cells	1-5 months	100% (n=24/24)	Lateral, 3th and 4th ventricles		[14]

CPT: Choroid plexus tumor (not necessary defined as CPC). MB: Medulloblastoma

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the choroid plexus of newborn mice led to aberrant proliferation and to the formation of fatal carcinoma within 1 to 5 months in 100% of the cases. These tumors recapitulate many features of their human counterparts, such as hydrocephalus, pleomorphic epithelioid cytology and prevalence in the lateral and the fourth ventricles. Genomic analysis revealed that, as in the model of Tong et al. (14), tumoral progression was associated to genomic instability and aberrant DNA metabolism, which may constitute hallmarks and key vulnerabilities of CPCs. In contrast to the models reported by Kawauchi et al. (15), medulloblastomas were never observed in the $Otx2^{CreER/+}$; $Rosa^{MycT58A/MycT58A}$; $Trp53^{fl/fl}$ model, despite expression of Otx2 in a large fraction of cerebellar granule cell precursors (GCPs), which constitute one of the best-characterised cell of origin for these tumors and the fact that MBs can be experimentally induced in mice by overexpressing Myc in GCPs. While further investigation will be required to understand why $Otx2^{CreER/+}$; $Rosa^{MycT58A/MycT58A}$; $Trp53^{fl/fl}$ mice exclusively develop CPCs, the exclusion of other neoplastic lesions makes it an ideal system to elucidate the mechanism of CPC formation (17). The temporal control offered by this model also opens up new opportunities to uncover unknown properties of these cancers, such as how the stage of induction of defined oncogenic alterations might influence later tumoral development. Finally, this novel animal model provides an invaluable tool to address the function of Otx2 itself in CPC tumorigenesis. Indeed, overexpression and focal gain of OTX2 were recently observed in cohorts of human plexus choroid tumors (18,19). OTX2 has been identified as an oncogene in the context of medulloblastomas, where it is frequently overexpressed (20-26) and might functionally interact with c-MYC (27). It is therefore conceivable that OTX2 could also play a role in CPC tumorigenesis and constitute a potential therapeutic target. Consistent with this hypothesis, Otx2 was shown to be required for both development and maintenance of choroid plexuses (28). Combination of the $Otx2^{CreET2R/+}$; $Rosa^{MycT58A/MycT58A}$; $Trp53^{fl/fl}$ model to the previously described Otx2fl/fl mouse line (29) now offers a unique opportunity to assess the function of Otx2 in choroid plexus oncogenesis.

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