Title: A polyomavirus-positive Merkel cell carcinoma mouse model supports a unified cancer origin

Authors: Wendy Yang[1]*, Sara Contente[1], Sarah Rahman[1]

Affiliations:
[1]Department of Pathology, Uniformed Services University of Health Sciences, Bethesda, MD 20814, USA.
*Corresponding author. Email: wendy.yang@usuhs.edu

Abstract:
The Germ Cell Theory, grounded in developmental biology, has enabled significant advances in understanding and treating germ cell tumors (GCTs), whereas somatic cancer research, dominated by the Somatic Mutation Theory (SMT), has stagnated and demands a paradigm shift. Accumulating evidence challenges the traditional separation between GCTs and somatic cancers. Polyomavirus-positive Merkel cell carcinoma (VP-MCC), an aggressive somatic cancer with exceptional genomic stability resembling GCTs yet paradoxical to SMT, suggested the potential for linking Germ Cell Theory to somatic oncogenesis. By inducing VP-MCC-like tumors from polyomavirus-transfected human primeval stem cells in mice, we directly established this link. This novel malignant somatic transformation model offers an unprecedented platform to dissect the molecular underpinning of VP-MCC carcinogenesis and advocates a developmental biology approach for somatic cancer research.

Main Text
Cancer has traditionally been classified into two primary groups based on presumed cellular origin: germ cell cancers (GCCs), arising from reproductive germline cells - also referred to as Type II GCTs - which constitute less than 1% of malignancies (*1*); and somatic cancers, originating from non-germline somatic cells, representing the vast majority of malignancies. Despite this traditional separation, all cancers share phenotypic hallmarks reminiscent of early embryonic/germ cell development, suggesting developmental arrest or "blocked ontogeny" (*2, 3*). This principle, encapsulated as "oncology recapitulates ontogeny," has significantly influenced GCC research and clinical practice yet remains largely overlooked in somatic cancer studies (*4, 5*), despite its clinical relevance to grading and prognosis (*6*).

The Germ Cell Theory, grounded in developmental biology, has yielded transformative insights into GCT biology and curative treatment strategies (*1, 4*). GCCs are genetically simple and epigenetically distinct (*1, 4*), originating from human primordial germ cells (hPGCs) (*1, 7*) - founder cells of the germline specified during weeks 2-3 of development (*8*). hPGCs exhibit latent pluripotency and intrinsic cancer stem cell-like properties, predisposing them to malignant transformation with minimal genetic alteration (*1*). In GCCs, transformed hPGCs and their totipotent derivatives - embryonal carcinoma (EC) cells thought to arise via parthenogenesis in vivo - constitute the cancer stem cell compartments of seminomas and non-seminomatous germ cell cancers (NSGCCs), respectively (*1*). Seminomas are composed entirely of transformed hPGCs, while NSGCCs such as ECs or malignant teratomas consist solely of EC cells or arise through multilineage somatic differentiation from ECs respectively (*1*).

Mounting biological and clinical evidence increasingly challenges the traditional separation of GCCs and somatic cancers and suggests a shared developmental origin. Notably, GCTs may occur as extragonadal GCTs (EGGCTs), demonstrate their capacity - like somatic cancers - to arise at any anatomical sites, albeit preferably along the body's midline (*9*). Approximately 3-6% of GCTs undergo malignant somatic transformation (MST) into somatic cancer phenotypes, often without additional mutations (*4, 10-12*). Many somatic cancers "aberrantly" express cancer-testis antigens normally restricted to germ cells (*13, 14*), and acquisition of a PGC-like state was shown to be essential for metastasis (*15*). Nevertheless, somatic cancer research remains dominated by the fragmented, mutation-centric SMT without a unifying biological framework or significantly improve patient outcomes (*5*). Although experimental models have shown a stem cell origin for somatic cancers (*2, 16*), their precise cellular identities remain undefined. Based on the substantial biological and clinical parallels between GCTs and somatic cancers, it appears rational to propose hPGC (or a hPGC-like state) as a shared stem cell origin for both. However, no experimental model has directly linked hPGC to somatic cancer via MST (*17*), despite the longstanding use of teratoma assays to study GCT development and tumorigenesis (*18*). These assays, which involve forming teratomas (a type of non-seminomatous GCT) in immunocompromised mice from injected human pluripotent stem cells (hPSCs), remain the gold standard for confirming pluripotency due to the intrinsic coupling of pluripotency and teratoma formation (*19*). While MST most commonly occurs in clinical teratomas, it has yet to be recapitulated in these models - suspected to result from the limited duration of in vivo assays compared to the prolonged latency typical of human disease (*17*).

Virus-positive Merkel cell carcinoma (VP-MCC), an aggressive neuroendocrine skin cancer, comprises ~80% of MCC cases and is defined by the presence of randomly and clonally integrated, truncated Merkel cell polyomavirus (MCPyV) genomes (~5 kb) (*20-22*). It presents two key features that seem to make it ideally suited for developing an MST model. First, VP-MCC exhibits extreme genomic stability, in stark contrast to virus-negative MCC (VN-MCC), which comprises the remaining ~20% and is among the most highly mutated human cancers. Similarly, other virus-associated cancers, such as HPV- and EBV-positive tumors, typically show higher mutational burdens than their VN counterparts (*23*). This exceptional genomic stability mirrors that of germ cell tumors (GCTs) and would enable the development of a genetically simple, experimentally tractable MST model. Although MCPyV is ubiquitous in normal skin flora, VP-MCC is very rare, and MCPyV transduction of cutaneous lineages fails to reproduce its molecular and histologic features - underscoring the paramount importance of cellular context, a principle also similar to GCT biology (*21, 22, 24*). Second, the small cell neuroendocrine carcinoma (SCNC) phenotype of MCC represents a poorly differentiated, aggressive end-stage state to which diverse somatic cancers converge upon progression (*25*). SCNCs, including MCC, also exhibit stem-like properties and multilineage differentiation potential (*26*), suggesting that VP-MCC is composed predominantly of somatic cancer stem cells, functionally analogous to seminomas or embryonal carcinomas (ECs) in GCTs. Together, these features support the potential of developing a VP-MCC-based MST model capable of capturing transformation events rapidly with immediate somatic differentiation arrests - within the temporal limits of mouse xenograft assays - where MST has otherwise not been observed (*17*). To directly test the Germ Cell Theory in somatic cancer, we proceeded to establish a mouse model based on the hypothesis that VP-MCC arises through MCPyV-driven MST of extragonadal hPGCs residing in the adult cutaneous niche. Supporting this, the skin is enriched in stem cell factor (SCF) and stromal cell-derived factor 1 (SDF-1), key regulators of hPGC

survival and chemotaxis (*27, 28*), while MCC's male predominance and predilection for the head and neck reflect Y chromosome-mediated hPGC viability and their preferential midline migratory trajectory (*1, 9*).

To investigate the proposed MCPyV-driven VP-MCC tumorigenic pathway, we designed a teratoma assay-like experiment aiming to prove MST from MCPyV transfected hPGCs to VP-MCC like tumors (VMLTs) in the adult mouse cutaneous niche. We transduced a truncated MCPyV genome (L82) via lentiviral vector into in vitro surrogates of hPGCs and additional two relevant hPSC types - human embryonic germ cells (hEGCs) and human embryonic stem cells (hESCs). Based on prior studies on hPSC-induced mouse teratoma xenografts, we estimated that three mice per condition would provide >99% success rate of forming mouse xenograft tumors (*29*). Eighteen adult NSG mice were divided into three groups (n = 6 per group). The **1st group** used **hPGC-like cells** (**hPGCLCs**) that are derived from hPSCs and recapitulate early-hPGCs (*30, 31*). Both normal hPGCs and hPGCLCs exhibit latent pluripotency - retaining germline unipotency in vivo without chimera or teratoma-forming capacity (*19, 32, 33*) - but can undergo parthenogenetic reversion to pluripotent hEGCs or hEGC-like cells (hEGCLCs) in vitro (*34, 35*). Due to limited culture yield, low-number (~$3x10^5$) cells of either hPGCLC_A4 (fig. S1A, provided by the Shioda lab, (*34*)) or MCPyV-transduced hPGCLC_A4_L82 (fig. S1B) were injected into the right flanks of three mice per condition. The **2nd group** used **Human induced pluripotent stem cells** (**hiPSCs**) that are derived from reprogramming of somatic cells and resemble hESCs (*36*). Based on our hypothesis, an indirect route from hPSCs to VP-MCC-like tumors (VMLTs) can theoretically be established through hPGCLC specification from competent hPSCs (*30*). Totipotent naïve-hESCs of the inner cell mass (ICM) at the early pre-implantation blastocyst stage and pluripotent formative-hESCs at the peri-implantation blastocyst stage are competent for hPGC induction, while more developmentally advanced pluripotent primed-hESCs of the late post-implantation blastocyst stage are not (*8, 30*). Only a small subset of conventionally cultured hESCs/hiPSCs maintain a naïve hESC state (*37*) while the vast majority remain at the primed hESC state (*38*), reflecting developmental plasticity between totipotent and primed hESC states (*1, 38*). Given that only a small subset of hiPSCs is capable of hPGCLC differentiation (*30, 37, 38*), we tested dose-dependent tumorigenicity by injecting hiPSC_A4_L82 (fig. S1C) at conventional-number (1×10^6) and high-number (2×10^7) cell doses into bilateral flanks of three mice per condition. The **3rd group** used **hEGC-like cells** (**hEGCLCs**) as the surrogate for hEGCs (*34, 35*), which are the in vitro parthenogenic derivatives of unipotent hPGCLCs and hPGCs respectively. Based on normal and teratoma developmental trajectories (*1*) - and given that VP-MCC is a somatic cancer - we considered the possibility that MST may require parthenogenetic reversion of a MCPyV-transformed hPGC to a pluripotent or totipotent intermediate (*1*). This would position hEGCs as a more direct candidate cell of origin than hPGCs, supporting the inclusion of hEGCLCs to test for direct conversion to VMLTs in vivo. It also remained unclear whether MCPyV transfected hEGCLC possess equivalent developmental potency to non-parthenogenetic hPSC counterparts such as MCPyV transfected hiPSCs, particularly in achieving a naïve state to support hPGCLC induction and thus an indirect MST route to VMLTs. To evaluate both possibilities, EGCLC_A4_L82 was injected at both cell doses (1×10^6 and 2×10^7) into bilateral flanks of three mice per condition. For both the 2nd group and the 3rd group, MCPyV-negative controls, hiPSC_A4 (provided by the Shioda lab, (*34*)) or hEGCLC_A4, were not included as it has been well established that pluripotency alone is coupled to teratoma formation without MST ((*17*); table S1).

Tumors comprised of both VMLT and teratoma components emerged exclusively from low-number hPGCLCs_L82 and from high-number hiPSC_L82 injections. Only teratoma component developed in conventional-number hiPSC_L82 mice, as well as hECLC_L82 mice regardless of cell numbers. No tumor formation was observed in MCPyV-negative hPGCLC control mice, consistent with their known "unipotency" in vivo from prior studies (*19, 33*). One of the three hPGCLC_A4_L82 mice (#86.1) failed to form any tumor, probably due to a technical failure of cell injection as the mouse was noted to be particularly challenging to apply physical restraint during injection (table S1). There was no gross or histological evidence of distant metastasis from any xenograft tumors with VMLTs. This finding is consistent with the known much less aggressive phenotype with only rare distant metastasis from VP-MCC tumor cell line-derived mouse tumors in prior studies (*39*). The reason for the marked difference of VP-MCC tumors' aggressiveness in human versus mouse is unknown and may reflect species differences.

Morphologic and Molecular Evidence for MST to VMLT and Independent Tumorigenic Pathways for VMLT and Teratoma Components in VMLT(+) Tumors

Gross findings support MST in all VMLT(+) tumors

Grossly, VMLT(+) tumors, which were composed of both VMLT and teratoma components, were markedly different from VMLT(-) tumors, which were composed only of a teratoma component. VMLT(+) tumors were solid and homogeneous with pale and friable texture grossly (Fig.1), while teratoma-only tumors were cystic and mucinous with heterogeneous discoloration (fig.S2). The finding mimicked the typical gross difference between clinical teratomas with MST and pure teratomas (*40*). The WHO criteria for MST in human GCTs require a minimum 5 mm expansion of pure somatic type of malignancy component (*41*). Our most salient tumor examples of MST to VMLT were T#85.4, consisting of two discrete tumor nodules with the larger nodule (T#85.4.N1 ~20 mm) composed of pure VMLT tissue (fig.S5A), and T#84.5R ~20 mm, composed almost entirely of VMLT (fig.S6A-B). The rest of the VMLTs also occupied substantial portions of tumors in sizable continuous sheets (~20% - ~80% of tumor masses) (fig.S7-8, fig.S6C, fig.S9A, fig.S20A).

VMLTs exhibited VP-MCC like histopathology

Histologic sections and cytology smears of all VMLTs showed sheets of immature small blue cells with either the typical MCC small round cell cytomorphology in the two hPGCLC_A4_L82 derived tumors T#85.3 or T#85.4 (fig.S5E, fig.S8E, fig.S11), or the atypical MCC large cell variant with admixed larger cells exhibiting prominent nucleoli and more abundant cytoplasm in all six high-number hiPSC_A4_L82 derived tumors, T#84.5L&R, T#85.1L&R, T#85.2L&R (fig.S6F, fig.S9D, fig.S10E, fig.S11). All VMLTs exhibited prominent histological characteristics of malignancy (fig.S12), including extracapsular invasion into dermis, with destruction of dermal striated muscle layer and lymphovascular invasion, with VMLT cells within lymphatic spaces (fig.S7, fig.S8A-D, fig.S6E, fig.S9B-C, fig.S10B-D).

VMLTs exhibited VP-MCC like molecular profiles

Molecular characterization by RT-qPCR (Fig.2, table S2) and/or immunohistochemical (IHC) studies (Fig.3, fig.S13, fig.S14-16, table S3) of VMLTs showed highly similar molecular profiles to each other and to the six VP-MCC cell lines (CVG-1, MKL-1, MKL-2, MS-1, PeTa and WaGa) (*42-48*). They were less similar to that of the two virus-negative variant MCC (VN-vMCC) cell lines (MCC-13 and UISO) (*47, 49, 50*) that do not express the key neuroendocrine lineage gene INSM1. The VMLTs showed mRNA and/or protein expression in neuroendocrine lineage genes, viral LT antigen, core pluripotency gene and PGC similar to that of VP-MCCs

with few exceptions, and a marked departure from that of the corresponding MCPyV transfected primeval stem cells, hPGCLC_A4_L82 or hiPSC_A4_L82 (Fig.2-3, fig.S13, fig.S14-16, table S2-3). The high Oct4 expression of all high-number hiPSC_A4_L82-derived VMLTs, in contrast to very low or absent expression in VP-MCC lines (fig.S17), raised concerns about a possible EC component in these VMLTs. However, it was ruled out by PLAP(-) and CD30(-) IHC stains on these VMLTs (fig.S14-16, table S3). Significantly lower ATOH1 mRNA levels were noted in all VMLTs than VP-MCC cell lines, which might be due to the incipient tumor status of VMLTs versus the advanced cancer stage of these VP-MCC cell lines (*51*).

Histological Spatial and Temporal Patterns of VMLT(+) Tumors Support Independent Tumorigenic Pathways for VMLT and Teratoma Components

All VMLT(+) tumors exhibited a distinct overall spatial pattern: primitive VMLT components localized superficially and peripherally, surrounding more differentiated teratoma components located deeper and centrally (fig. S6C, S7, S9A, S10A, S18). This organization contrasts with the typical "differentiation gradient" of hPSC-derived mouse xenograft teratomas, which display a less differentiated core and more mature peripheral regions (*19*). Notably, the teratoma components within VMLT(+) tumors did retain the expected maturation gradient of xenograft teratomas (fig. S18A–B). The opposing spatial arrangements of the overall VMLT(+) tumors versus their teratoma components support the conclusion that VMLT tumorigenesis occurs via a distinct, independent pathway that requires proximity to the cutaneous microenvironment.

Bilateral tumors of high-number hiPSC_A4_L82 mouse#85.2 were only allowed to grow for a short period of time (26 days, table S1). However, a discrete small VMLT component (<20-30% of tumor volume) was already present in part of superficial dermis in T#85.2L or T#85.2R, before the VMLT component grew to be the dominant component (~70-~100%) at later time points (54 days and 77 days) in the other two very high number hiPSC_A4_L82 mouse#84.5 and mouse#85.1.

DNA Demethylation Signatures Support Direct MST from an Obligatory Late-hPGC State to VP-MCC Without a Pluripotent or Totipotent Intermediate

DNA methylation, though dynamic during development, serves as a relatively stable epigenetic marker that retains lineage memory. Cancer cells may maintain DNA methylation signatures reflective of their cell of origin despite reprogramming. In human development, only two waves of global DNA demethylation occur, each producing a transient hypomethylation nadir. The first occurs in totipotent naïve hESC/ICM stage at ~40% global DNA methylation (GDM); the second - and lowest - in late hPGC stage at ~6% GDM ((*52*, *53*); fig. S19). This extreme low GDM is unique to late hPGCs and no further global demethylation occurs beyond these early stages. In vitro models mirror these states: hiPSCs and hEGCLCs (primed hESC-like) show high GDM at ~70%, hPGCLCs (early hPGC-like) show intermediate GDM (~50%) ((*34*); fig. S19). GCCs reflect this epigenetic hierarchy: seminomas match the extreme global DNA hypomethylation of late hPGCs, ECs show moderate global DNA hypomethylation of the naïve hESCs, and teratomas typically exhibit higher GDM due to somatic differentiation (Fig. 5). All GCCs thus retain variable degrees of global DNA hypomethylation consistent with late hPGC origin. Somatic cancers are also known to exhibit global DNA hypomethylation, positively correlating with degree of malignancy and cancer progression (*54*). SCNCs, including MCC, share marked global DNA hypomethylation (*55*, *56*) with seminomas and ECs - the stem-like compartments of GCCs - as well as stemness and developmental plasticity. To investigate further, we compared GDM in VMLTs, MCC lines, and the seminoma cell line TCam-2 (*57*).

One caveat is that DNA methylation is sensitive to microenvironment, and tumor cells cultured in vitro often exhibit artificially elevated GDM (*58*). In vitro culture can elevate GDM of TCam-2, which, despite its late-hPGC origin, shows moderate GDM - similar to EC lines - rather than the extreme hypomethylation of primary seminomas ((*59*) *and personal communications*).

Global 5mC Levels in hiPSC_A4_L82 and TCam-2 Are Consistent with Known GDM Profiles
Semi-quantitative flow cytometry analysis of global 5-methylcytosine (5mC) revealed that the hiPSC_A4_L82 line, modeling the primed hESC state, exhibited markedly higher 5mC levels (MFI ~113K) compared to the seminoma cell line TCam-2 (MFI ~7K), which is known to display moderate DNA hypomethylation (Fig. 4; Table S4).

Extreme Global DNA Hypomethylation in VMLTs Suggest Direct MST from an Obligatory Late-hPGC State Without a Pluripotent or Totipotent Intermediate
Flow cytometry analysis of VMLT tumor cells revealed extremely low global 5mC levels (MFI: ~2K–~3.5K), approaching those of isotype negative controls (MFI: ~2K-~3K) (Fig. 4; table S4). These results indicate near-complete DNA methylation erasure, a state physiologically restricted to late-hPGCs (fig. S19; Fig. 5). VMLTs were derived either from hPGCLC_A4_L82 (modeling early hPGCs with ~50% GDM), or from hiPSC_A4_L82 (modeling primed hESCs with ~70% GDM) (*35*), confirming a profound GDM reset during transformation.

VMLTs retain the extreme global DNA hypomethylation signature unique to the “late-hPGC” state, suggesting a model in which transformation occurs directly from this state across the Weismann Barrier (WB), bypassing any intermediary stage typically marked by moderate global DNA hypomethylation (Fig. 5). This epigenetic distinction enables delineation of the tumorigenic pathways for VMLTs derived from hPGCLC_A4_L82 versus those derived from high-number hiPSC_A4_L82 cells. A direct pathway, for hPGCLC_A4_L82-derived VMLTs, involved further germline progression from an early hPGC-like state (modeled by hPGCLC_A4_L82) to the requisite “late-hPGC” state, followed by MST to VMLTs (Fig. 5). In contrast, the indirect pathway, for hiPSC_A4_L82-derived VMLT, necessitated an extra initial hPGCLC specification step, in which a small subset of naïve hiPSC_A4_L82 cells first differentiates into hPGCLC_A4_L82-like cells. These then proceed to the “late-hPGC” state before undergoing MST to VMLTs (Fig. 5). The observed dosage effect - VMLT formation in high-number but not conventional-number hiPSC_A4_L82 injections, with no such effect in low-number hPGCLC_A4_L82-derived tumors - can be attributed to this additional hPGCLC specification bottleneck. Moreover, clinical reports of rare MST from pure seminomas to somatic malignancies (*60*) offer further clinical support for the plausibility of direct MST from a “late-hPGC”-like state to somatic cancer, reinforcing the pathway proposed here.

Based on the VMLT tumorigenic pathway supported by the unique epigenetic signature of “late-hPGC” and the known intrinsic pluripotency-teratoma coupling, an independent tumorigenic pathway is suggested for the teratoma components of hPGCLC_A4_L82 derived VMLT(+) tumors, involving survival of hPGCLC_A4_L82 in vivo with subsequent parthenogenic conversion to a pluripotent intermediate in vivo followed by multi-lineage somatic differentiation ((*19*), Fig.5). Similarly, an independent tumorigenic pathway is also suggested for the teratoma components of the high-number hiPSC_A4_L82 derived VMLT(+) tumors, involving multi-lineage somatic differentiation from the pluripotent hiPSC_A4_L82 (Fig.5). This

independent tumorigenic pathway to the teratoma component can explain only teratoma formation without VMLT components in conventional-number hiPSC_A4_L82 mice.

The independent tumorigenic pathways for the VMLT component versus the teratoma component can also provide the rationale for the co-presence of VMLT and teratoma components in this VP-MCC mouse xenograft model, while no mixed VP-MCC and teratoma tumor has been reported in clinical settings. The answers lie in the fact that the experimental mouse teratomas derived from hPSCs are known to be polyclonal (*19*), while clinical cancer is a clonal disease originated from a single transformed cell including VP-MCC that is characterized by a clonal viral insertion site (*20*).

MCC cell lines exhibited global DNA hypomethylation similar to seminoma cell line TCam2

To support our model of VP-MCC tumorigenesis via direct transformation from an obligatory "late-hPGC" state, we analyzed global 5mC levels in six VP-MCC cell lines and compared them to the only validated seminoma cell line TCam-2, which consists of transformed late-hPGCs (*1*). Flow cytometry showed that VP-MCC lines exhibited moderate global DNA hypomethylation (MFI: ~5K-16K), similar to TCam-2 (~7K), though slightly higher than VPMLs (~2K-3.5K) (Fig. 4; Table S4). As both VP-MCC lines and TCam-2 were cultured under identical conditions (RPMI + 10% FBS), their moderately elevated 5mC levels relative to that of VMLTs ($p < 0.027$) likely reflect epigenetic drift due to in vitro culture and passage (*58*).

Interestingly, the two VN-vMCC cell lines, MCC-13 and UISO, grown under the same culture condition also displayed comparable levels of moderate hypomethylation (MFL: ~7K-~9K) to that of VP-MCC cell lines, aligning with previous findings (*55, 56*). The similarity in GDM between VN-vMCC lines and TCam-2 under matched culture conditions suggests that primary VN-vMCC tumors may also exhibit extreme hypomethylation - an epigenetic hallmark of the "late-hPGC" state observed in primary seminomas. In the case of VN-vMCCs, this "late-hPGC"-like state may arise via reprogramming from somatic cells driven by extensive somatic mutations, including pRB and p53 mutations, characteristic of these tumors (*15, 22, 56*) (Fig. 5; table S4).

Failure of McPyv Transfected hEGCLCs to Form VMLT Provides Functional Evidence Supporting VMLT Tumorigenesis Without a Parthenogenetic Pluripotent or Totipotent Intermediate

Failure of hEGCLC_A4_L82 to form VMLTs supports VMLT tumorigenesis without a parthenogenetic pluripotent intermediate

Only teratomas, and no VMLT components, were observed in all tumors derived from pluripotent hEGCLC_A4_L82, indicating a lack of direct conversion to VMLTs in vivo from hEGCLC_A4_L82 - the in vitro pluripotent derivative of hPGCLC_A4_L82. This finding therefore provides functional evidence opposing the involvement of a parthenogenetic pluripotent hESC-like intermediate in MST to VMLTs, and further supports a VMLT tumorigenic pathway that is independent of teratoma formation and uncoupled from parthenogenetic pluripotency.

Failure of high-number hEGCLC_A4_L82 cells to form VMLTs in vivo supports the absence of a parthenogenetic totipotent intermediate in the formation of VMLT or teratoma components

Previous studies indicate that the developmental potency of parthenogenetic derivatives of normal hPGCs, such as hEGCLCs or hEGCs, decreases along the hPGC developmental trajectory: hEGCs derived from post-migratory late-hPGCs lose bona fide pluripotency, whereas hEGCLCs originating from hPGCLCs (modeling early hPGCs) regain pluripotency, as demonstrated by their inability or ability to form teratomas in vivo, respectively (*19, 34*). Thus, prior entry into the germline and a corresponding decline in developmental potency define the normal hPGC parthenogenesis paradigm. In contrast, transformed hPGCs in NSGCCs exhibit a reversed trajectory, with parthenogenetic derivatives showing increased developmental potency along germline progression: transformed late-stage hPGCs give rise to totipotent EC cells, while transformed early-stage hPGCs yield less potent, primed-state hESC-like derivatives. In this transformed paradigm, prior germline entry is associated with an upregulation of developmental potency - a distinct hallmark of NSGCC transformation ((*1*) *and personal communications*).

The inability of high-number hEGCLC_A4_L82 cells to form VMLTs in mice, compared to the successful VMLT formation by high-number hiPSC_A4_L82 cells, provides functional evidence that hEGCLC_A4_L82 failed to transition to the totipotent naïve hESC-like state required for VMLT induction via the indirect pathway through hPGCLC_A4_L82 specification. This reduced potency relative to its non-parthenogenetic counterpart aligns with the normal, rather than NSCGC-transformed, hPGC parthenogenesis paradigm. This observation reflects MCPyV's inability to functionally enhance parthenogenetic potency in hPGCLCs_A4_L82, in contrast to the developmental potency upregulation characteristic of NSGCC transformation. MCPyV's apparent incapacity to induce NSGCC-like transformation indicates that hPGCLC_A4_L82 cells, upon viral transduction, cannot parthenogenetically acquire a totipotent EC-like state. These findings are consistent with the GDM evidence supporting the requirement of a "late hPGC" state before direct MST to VMLTs, without the involvement of a totipotent parthenogenetic intermediate. Furthermore, MCPyV's inability to induce NSGCT-like transformation suggests that the teratoma components observed in hPGCLC_A4_L82-derived VMLT(+) tumors have arisen directly from hPGCLC_A4_L82 cells modeling early-hPGCs, without germline developmental progression in vivo to a "late-hPGC" state.

Discussion

The inability of hPGCLC_A4 to form any tumor - teratoma or VMLT - compared to the formation of both components by hPGCLC_A4_L82 implicates several potential tumorigenic functions of MCPyV. These include: (i) promoting survival or anti-apoptotic activity in hPGCLC_A4_L82 (contributing to both VMLTs and teratomas); (ii) enabling parthenogenetic reacquisition of pluripotency (teratomas only); (iii) facilitating progression from an early- to late-hPGC state (VMLTs only); and (iv) directly transforming late-hPGC-like cells into VMLTs (VMLTs only). Given MCPyV's inability to induce NSGCT-like transformation and the established teratoma-forming capacity of normal hEGCLCs, its role in teratoma formation is likely minimal. Rather, as extragonadal hPGCs are capable of spontaneous germline differentiation into late-hPGCs or beyond in non-gonadal environments before undergoing apoptosis (*1*), the most plausible MCPyV-driven mechanisms are enhanced survival and direct transformation of late-hPGC-like cells into VMLTs. Further studies are warranted to elucidate the molecular mechanisms underlying MCPyV-mediated VMLT formation.

In summary, we developed an MST mouse model demonstrating MCPyV-driven transformation of hPGCLCs into somatic VMLTs. GDM analyses identified a "late-hPGC" state as a necessary developmental precursor for direct MST to VMLTs. Temporal, spatial, epigenetic, and

functional data collectively support the existence of independent tumorigenic trajectories for the teratoma and VMLT components within the same VMLT(+) tumor, with no evidence for a parthenogenetic pluripotent or totipotent intermediate in VMLT tumorigenesis. This model thereby establishes a direct developmental link between late-hPGCs and a somatic cancer.

Notably, the late-hPGC state is also a requisite intermediate in malignant germ cell cancers (GCCs) (*1*), indicating overlapping cancer biology between germ cell and somatic malignancies. This convergence is exemplified by the globally hypomethylated epigenetic profile characteristic of late-hPGCs - a recognized molecular hallmark observed across both cancer categories (*1, 54*). Our model supports a fundamental divergence between normal human development and VP-MCC tumorigenesis at the level of the Weismann Barrier (WB). In canonical development, pluripotent hESCs generate somatic tissues through orderly, multi-lineage differentiation. In contrast, VP-MCC appears to originate from a “unipotent” late-hPGC with intrinsic germline suppression of somatic fate, yet bearing latent or poised pluripotency (*32*), that aberrantly crosses the WB into the somatic compartment, differentiating exclusively along a neuroendocrine lineage and undergoing developmental arrest. These findings suggest that VP-MCC may arise via an aberrant ontogenic process in which the germline directly gives rise to a somatic malignancy - akin to the emergence of a highly abnormal new life form. This MST model also supports key distinctions between somatic cancers such as VP-MCC and GCCs exhibiting multilineage somatic differentiation, namely malignant teratomas. The latter more closely parallels normal human embryogenesis, requiring a totipotent embryonal carcinoma (EC) cell intermediate to traverse the Weismann barrier and undergo multilineage somatic differentiation with aberrant developmental arrest. These EC cells are thought to arise via parthenogenetic transformation of late-stage hPGCs in vivo, rather than from fertilization-derived zygotes. Future investigations are needed to determine whether this unifying germ cell-based paradigm applies more broadly to other somatic malignancies. For instance, in VN-vMCCs, somatic mutations may induce a functionally equivalent “late-hPGC” state, substituting for the native “late-hPGC” state observed in VP-MCCs.

Finally, the genetic simplicity and experimental tractability of our VP-MCC–based MST model provide a robust platform for elucidating the molecular mechanisms driving VP-MCC pathogenesis. This system may also yield critical insights into the molecular underpinnings of VN-MCC and other treatment-refractory high-grade neuroendocrine carcinomas (HGNECs) characterized by high mutational burdens. By directly establishing a developmental origin for VP-MCC, this model supports a shift toward a developmental biology–driven framework for advancing somatic cancer research.

References and Notes

1. J. W. Oosterhuis, L. H. J. Looijenga, Human germ cell tumours from a developmental perspective. *Nature Reviews Cancer* **19**, 522-537 (2019).
2. D. Hanahan, R. A. Weinberg, Hallmarks of cancer: the next generation. *Cell* **144**, 646-674 (2011).
3. S. Sell, G. B. Pierce, Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. *Lab Invest* **70**, 6-22 (1994).
4. S. M. Tu, L. L. Pisters, Curing Cancer: Lessons from a Prototype. *Cancers (Basel)* **13**, (2021).

5. S. Huang, A. M. Soto, C. Sonnenschein, The end of the genetic paradigm of cancer. *PLoS Biol* **23**, e3003052 (2025).
6. A. Jögi, M. Vaapil, M. Johansson, S. Påhlman, Cancer cell differentiation heterogeneity and aggressive behavior in solid tumors. *Ups J Med Sci* **117**, 217-224 (2012).
7. J. Taguchi *et al.*, DMRT1-mediated reprogramming drives development of cancer resembling human germ cell tumors with features of totipotency. *Nat Commun* **12**, 5041 (2021).
8. D. Chen *et al.*, Human Primordial Germ Cells Are Specified from Lineage-Primed Progenitors. *Cell Rep* **29**, 4568-4582 e4565 (2019).
9. M. De Felici *et al.*, To Be or Not to Be a Germ Cell: The Extragonadal Germ Cell Tumor Paradigm. *Int J Mol Sci* **22**, (2021).
10. J. B. Kum *et al.*, Molecular genetic evidence supporting the origin of somatic-type malignancy and teratoma from the same progenitor cell. *Am J Surg Pathol* **36**, 1849-1856 (2012).
11. J. Taylor *et al.*, Germ cell tumors and associated hematologic malignancies evolve from a common shared precursor. *J Clin Invest* **130**, 6668-6676 (2020).
12. N. Wyvekens *et al.*, Molecular correlates of male germ cell tumors with overgrowth of components resembling somatic malignancies. *Mod Pathol* **35**, 1966-1973 (2022).
13. D. S. Nin, L. W. Deng, Biology of Cancer-Testis Antigens and Their Therapeutic Implications in Cancer. *Cells* **12**, (2023).
14. A. J. Simpson, O. L. Caballero, A. Jungbluth, Y. T. Chen, L. J. Old, Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer* **5**, 615-625 (2005).
15. Z. Ma, F. Zhang, A. Liu, H. Lin, C. Liu, Somatic-to-primordial germ cell-like transformation is critical in tumor initiation of mouse breast tumor 4T1 cells. *Am J Cancer Res* **13**, 1471-1485 (2023).
16. J. E. Visvader, G. J. Lindeman, Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* **8**, 755-768 (2008).
17. I. Damjanov, P. W. Andrews, Teratomas produced from human pluripotent stem cells xenografted into immunodeficient mice - a histopathology atlas. *Int J Dev Biol* **60**, 337-419 (2016).
18. J. Montilla-Rojo *et al.*, Teratoma Assay for Testing Pluripotency and Malignancy of Stem Cells: Insufficient Reporting and Uptake of Animal-Free Methods-A Systematic Review. *Int J Mol Sci* **24**, (2023).
19. M. Aleckovic, C. Simon, Is teratoma formation in stem cell research a characterization tool or a window to developmental biology? *Reprod Biomed Online* **17**, 270-280 (2008).
20. H. Feng, M. Shuda, Y. Chang, P. S. Moore, Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* **319**, 1096-1100 (2008).
21. P. W. Harms *et al.*, The biology and treatment of Merkel cell carcinoma: current understanding and research priorities. *Nat Rev Clin Oncol* **15**, 763-776 (2018).
22. E. A. Pedersen, M. E. Verhaegen, M. K. Joseph, K. L. Harms, P. W. Harms, Merkel cell carcinoma: updates in tumor biology, emerging therapies, and preclinical models. *Front Oncol* **14**, 1413793 (2024).
23. Z. Lu, P. Fan, W. Huo, Y. Feng, R. Wang, Corrigendum to "Genomic profiles and their relationships with clinical characteristics and immune features in cervical cancer" [Translational Oncology 44 (2024) 1-10/101923]. *Transl Oncol* **47**, 102037 (2024).
24. T. Kervarrec *et al.*, Histogenesis of Merkel Cell Carcinoma: A Comprehensive Review. *Front Oncol* **9**, 451 (2019).

25. N. G. Balanis *et al.*, Pan-cancer Convergence to a Small-Cell Neuroendocrine Phenotype that Shares Susceptibilities with Hematological Malignancies. *Cancer Cell* **36**, 17-34.e17 (2019).
26. Z. Zhang *et al.*, Stemness and inducing differentiation of small cell lung cancer NCI-H446 cells. *Cell Death Dis* **4**, e633 (2013).
27. B. M. Foster, K. L. Langsten, A. Mansour, L. Shi, B. A. Kerr, Tissue distribution of stem cell factor in adults. *Exp Mol Pathol* **122**, 104678 (2021).
28. C. Quan *et al.*, Dermal fibroblast expression of stromal cell-derived factor-1 (SDF-1) promotes epidermal keratinocyte proliferation in normal and diseased skin. *Protein Cell* **6**, 890-903 (2015).
29. T. A. Prokhorova *et al.*, Teratoma formation by human embryonic stem cells is site dependent and enhanced by the presence of Matrigel. *Stem Cells Dev* **18**, 47-54 (2009).
30. N. Irie *et al.*, SOX17 is a critical specifier of human primordial germ cell fate. *Cell* **160**, 253-268 (2015).
31. K. Sasaki *et al.*, Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells. *Cell Stem Cell* **17**, 178-194 (2015).
32. H. G. Leitch, A. Smith, The mammalian germline as a pluripotency cycle. *Development* **140**, 2495-2501 (2013).
33. F. Sugawa *et al.*, Human primordial germ cell commitment in vitro associates with a unique PRDM14 expression profile. *EMBO J* **34**, 1009-1024 (2015).
34. M. Kobayashi *et al.*, Expanding homogeneous culture of human primordial germ cell-like cells maintaining germline features without serum or feeder layers. *Stem Cell Reports* **17**, 507-521 (2022).
35. M. J. Shamblott *et al.*, Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci U S A* **95**, 13726-13731 (1998).
36. K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676 (2006).
37. J. K. Cinkornpumin *et al.*, Naive Human Embryonic Stem Cells Can Give Rise to Cells with a Trophoblast-like Transcriptome and Methylome. *Stem Cell Reports* **15**, 198-213 (2020).
38. T. Messmer *et al.*, Transcriptional Heterogeneity in Naive and Primed Human Pluripotent Stem Cells at Single-Cell Resolution. *Cell Rep* **26**, 815-824 e814 (2019).
39. L. R. Dresang *et al.*, Response of Merkel cell polyomavirus-positive merkel cell carcinoma xenografts to a survivin inhibitor. *PLoS One* **8**, e80543 (2013).
40. T. M. Ulbright, Germ cell tumors of the gonads: a selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. *Mod Pathol* **18 Suppl 2**, S61-79 (2005).
41. D. M. Berney *et al.*, An introduction to the WHO 5th edition 2022 classification of testicular tumours. *Histopathology* **81**, 459-466 (2022).
42. A. Guastafierro *et al.*, Characterization of an early passage Merkel cell polyomavirus-positive Merkel cell carcinoma cell line, MS-1, and its growth in NOD scid gamma mice. *J Virol Methods* **187**, 6-14 (2013).
43. R. Houben *et al.*, Mechanisms of p53 restriction in Merkel cell carcinoma cells are independent of the Merkel cell polyoma virus T antigens. *J Invest Dermatol* **133**, 2453-2460 (2013).
44. R. Houben *et al.*, Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. *J Virol* **84**, 7064-7072 (2010).

45. E. M. Martin *et al.*, Parathyroid hormone-related protein, chromogranin A, and calcitonin gene products in the neuroendocrine skin carcinoma cell lines MKL1 and MKL2. *Bone Miner* **14**, 113-120 (1991).
46. S. T. Rosen *et al.*, Establishment and characterization of a neuroendocrine skin carcinoma cell line. *Lab Invest* **56**, 302-312 (1987).
47. M. Shuda *et al.*, T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc Natl Acad Sci U S A* **105**, 16272-16277 (2008).
48. C. Velásquez *et al.*, Characterization of a Merkel Cell Polyomavirus-Positive Merkel Cell Carcinoma Cell Line CVG-1. *Front Microbiol* **9**, 713 (2018).
49. S. G. Ronan, A. D. Green, A. Shilkaitis, T. S. Huang, T. K. Das Gupta, Merkel cell carcinoma: in vitro and in vivo characteristics of a new cell line. *J Am Acad Dermatol* **29**, 715-722 (1993).
50. J. A. Thomson, J. H. Leonard, K. McGregor, R. A. Sturm, P. G. Parsons, A nonconsensus octamer-recognition sequence (TAATGARAT-motif) identifies a novel DNA binding protein in human Merkel cell carcinoma cell lines. *Int J Cancer* **58**, 285-290 (1994).
51. T. Gambichler *et al.*, Prognostic relevance of high atonal homolog-1 expression in Merkel cell carcinoma. *J Cancer Res Clin Oncol* **143**, 43-49 (2017).
52. H. Guo *et al.*, The DNA methylation landscape of human early embryos. *Nature* **511**, 606-610 (2014).
53. L. Wen, F. Tang, Human Germline Cell Development: from the Perspective of Single-Cell Sequencing. *Mol Cell* **76**, 320-328 (2019).
54. M. Ehrlich, DNA hypomethylation in cancer cells. *Epigenomics* **1**, 239-259 (2009).
55. J. Gravemeyer *et al.*, DNA-methylation patterns imply a common cellular origin of virus- and UV-associated Merkel cell carcinoma. *Oncogene* **41**, 37-45 (2022).
56. P. W. Harms *et al.*, Viral Status Predicts the Patterns of Genome Methylation and Decitabine Response in Merkel Cell Carcinoma. *J Invest Dermatol* **142**, 641-652 (2022).
57. Y. Mizuno, A. Gotoh, S. Kamidono, S. Kitazawa, [Establishment and characterization of a new human testicular germ cell tumor cell line (TCam-2)]. *Nihon Hinyokika Gakkai Zasshi* **84**, 1211-1218 (1993).
58. D. J. Smiraglia *et al.*, Excessive CpG island hypermethylation in cancer cell lines versus primary human malignancies. *Hum Mol Genet* **10**, 1413-1419 (2001).
59. D. Nettersheim *et al.*, Analysis of TET expression/activity and 5mC oxidation during normal and malignant germ cell development. *PLoS One* **8**, e82881 (2013).
60. K. P. Dieckmann *et al.*, Pure Testicular Seminoma Relapsing Late with Somatic Type Malignancy. *Case Rep Oncol Med* **2017**, 2457023 (2017).

Acknowledgments:

We would like to thank Toshi Shioda for providing LTC-hPGCLC_A4 and hiPSC_A4 cell lines, technical guidance and protocols; Yuan Chang, Patrick Moore, Masahiro Shuda, Isaac Brownell and James DeCaprio for providing virus-positive and virus-negative Merkel cell carcinoma cell lines, technical guidance, and/or protocols; Alan Epstein for providing TCam2 cell line and protocols; Reety Arora, Sudhir Krishna, Eric Campeau and Paul Kaufman for AddGene plasmids; Joel Moncur for mouse xenograft tumor histopathological evaluation and manuscript review; Guanghua Wang for cell line and mouse xenograft tumor authentication, mouse tumor histopathological and manuscript review; Geeta Upadhyay for technical advices and manuscript review; Steve Dumler and Dennis Grab for manuscript review; as well as the USUHS Genomics

Core and Flow Cytometry Core Lab in Biomedical Instrumentation Center for DNA primer synthesis and flow cytometry services respectively.

Funding: USUHS StartUp Fund

Author contributions:

Conceptualization: WY

Methodology: WY

Investigation: WY, SR

Visualization: SC, WY

Funding acquisition: WY

Project administration: WY

Supervision: WY

Writing - original draft: WY

Writing - review & editing: WY, SC

Competing interests: Authors declare that they have no competing interests.

Data and materials availability: hPGCLC_A4 and hiPSC_A4 cell lines and their derivative cell lines are subject to the restrictions stated in a materials transfer agreement (MTA) with Dr. Toshi Shioda lab at Massachusetts General Hospital. All data are available in the main text or the supplementary materials.

Disclaimer

The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of Defense.

Supplementary Materials

Materials and Methods

Figs. S1 to S19

Tables S1 to S6

References (*#61*)

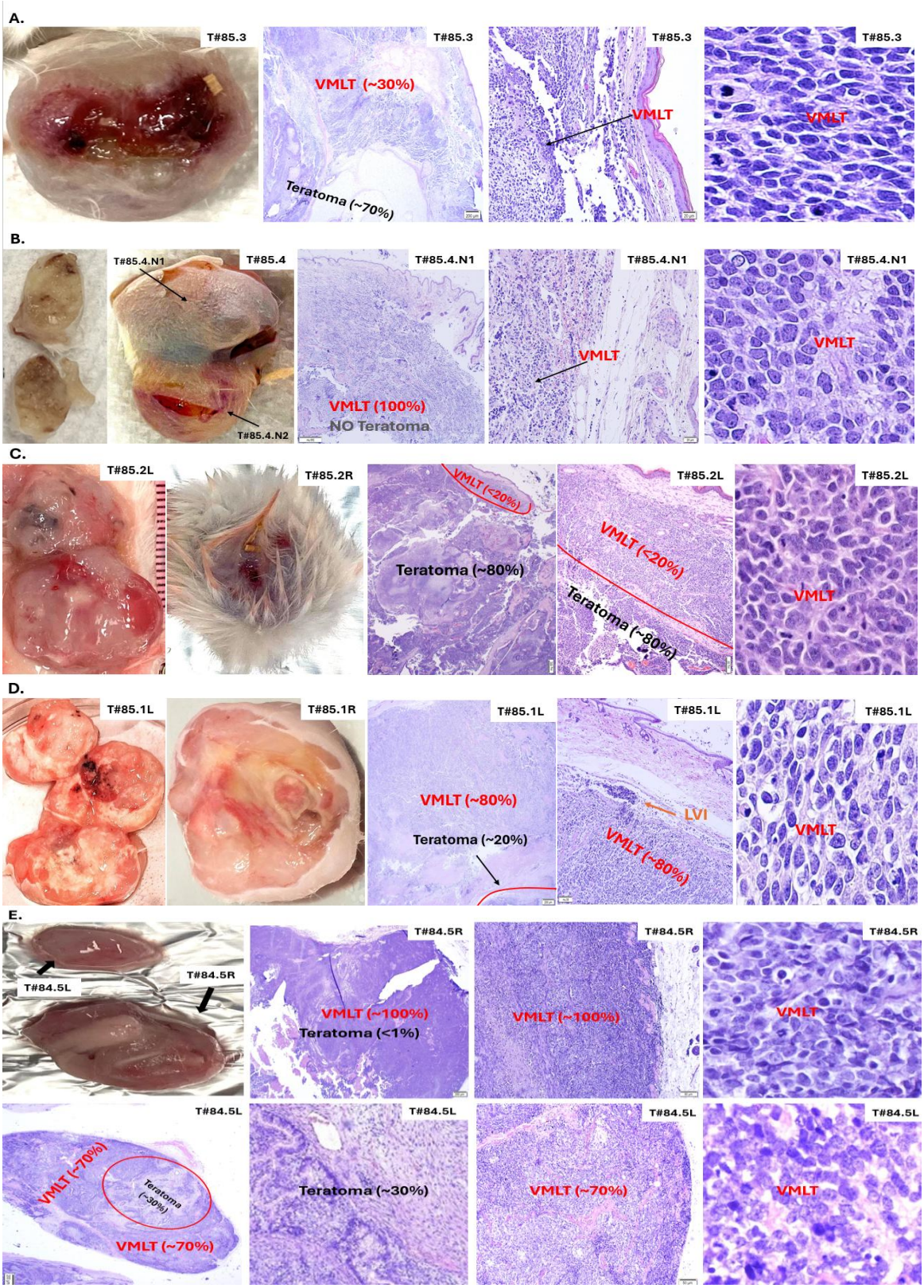

Fig. 1. Gross appearance and representative histology of mouse VP-MCC-like tumors (VMLTs) derived from human primeval stem cells: Injected with hPGCLC_A4_L82 (~ 3×10^5 cells): **(A)** Mouse #85.3. **(B)** Mouse #85.4. Injected with hiPSC_A4_L82 (~ 2×10^7 cells): **(C)** Mouse # 85.2. (**D)** Mouse #85.1 (**E)** Mouse #84.5.

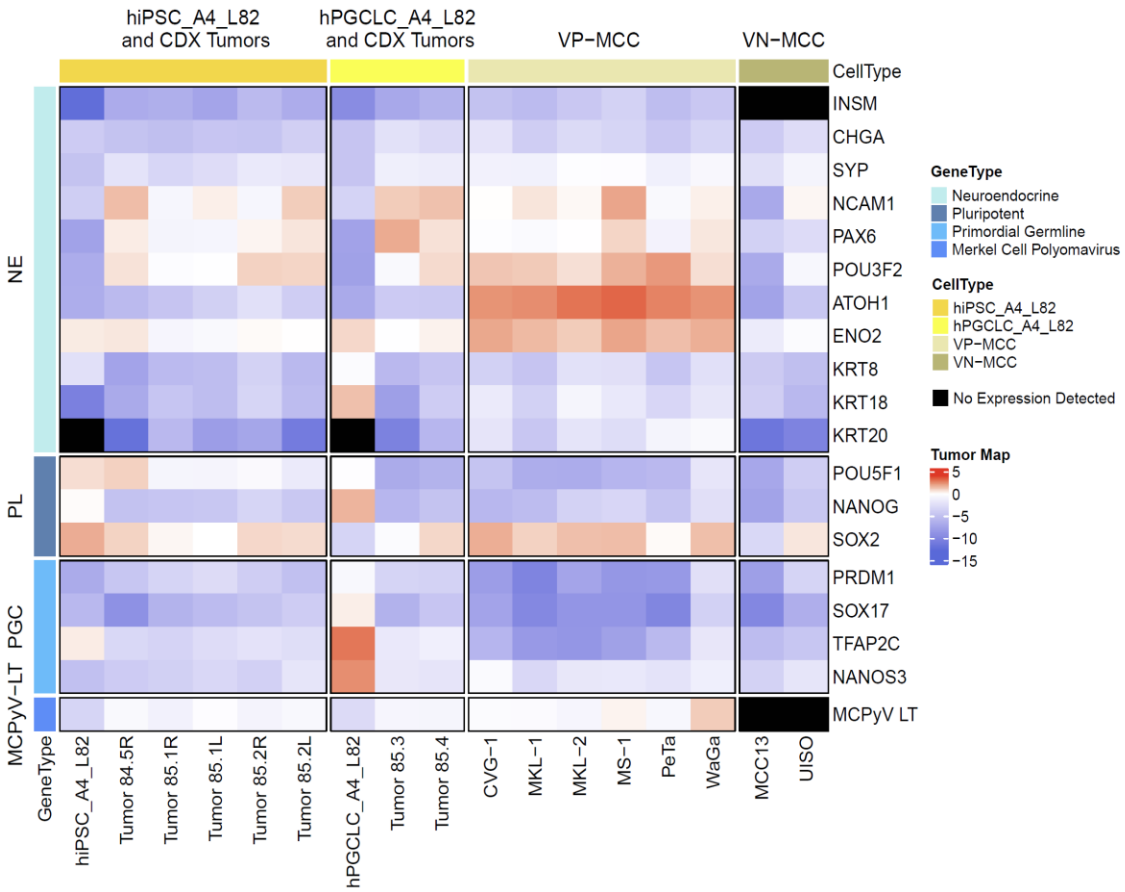

Fig. 2. Heatmap of mRNA expression. Comparison of mRNA expression of key neuroendocrine lineage genes, core pluripotent genes, PGC marker genes and McPyv LT viral oncogene among transfected primeval stem cell lines (hiPSC_A4_L82 and hPGCLC_A4_L82), their derivative mouse CDX tumors, VP-MCC and VN-MCC cell lines.

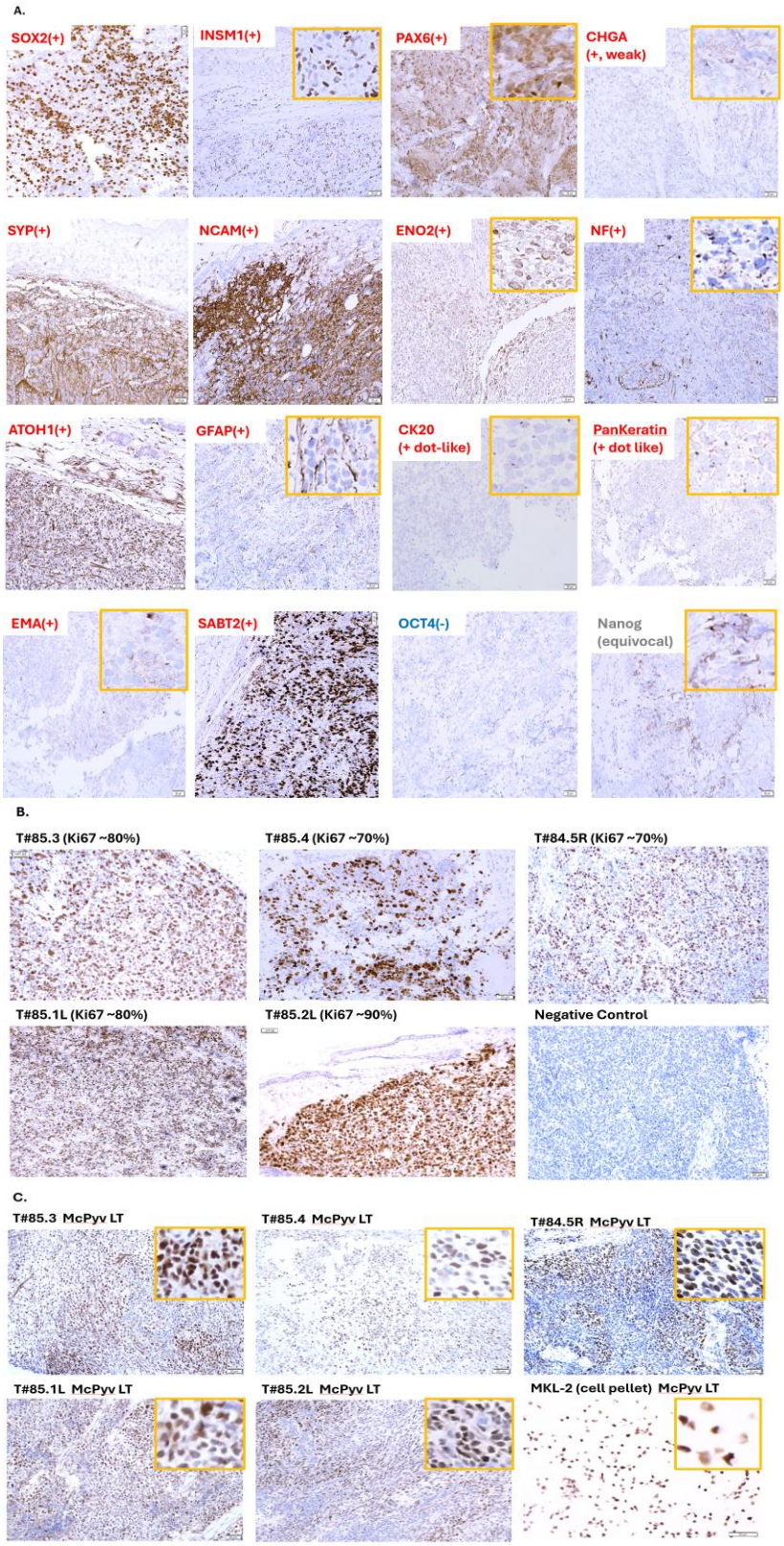

Fig. 3. Immunohistochemical studies of mouse VMLTs. (A) Protein expression of a panel of genes in VMLT of T#85.4.N1. **(B)** High Ki67 proliferative indexes of VMLTs from five mice. **(C)** Positive expression of McPyv LT oncoprotein in VMLT from five mice. MKL-2 cell pellet was used as a positive control.

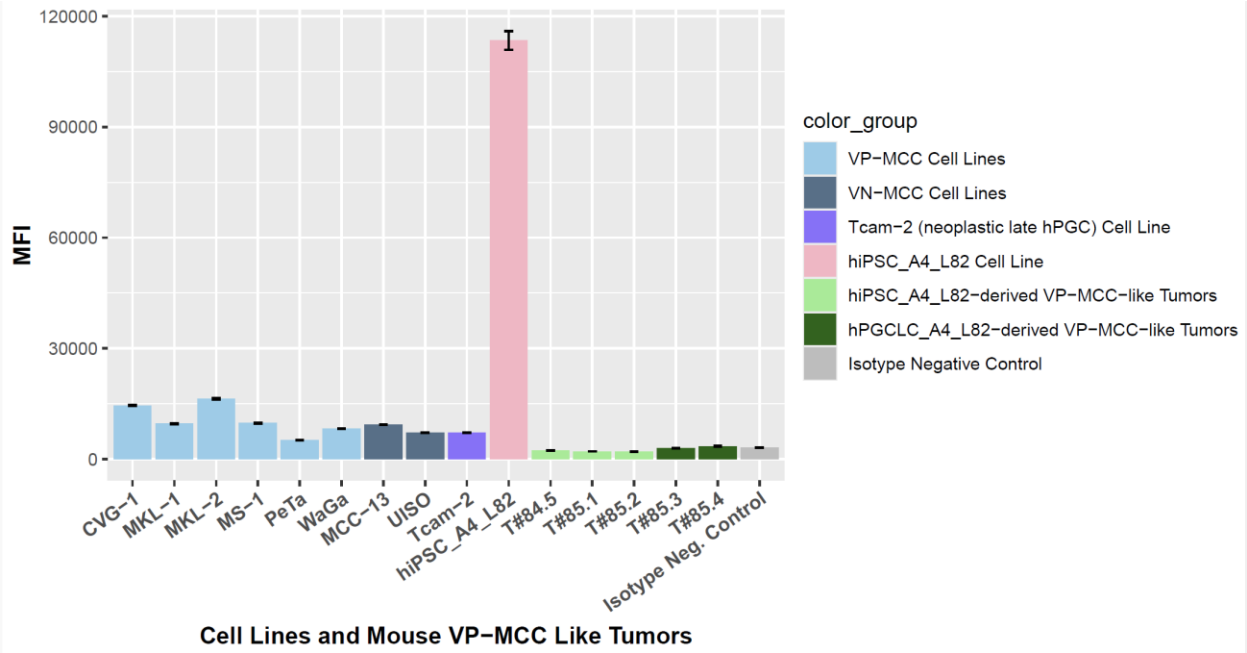

Fig. 4. Global 5mC levels by flow cytometry. Flow cytometry estimated global 5mC levels semi-quantitively of six VP-MCC cell lines, two VN-MCC cell lines, the seminoma Tcam-2 cell line, hiPSC_A4_L82 cell line, hEGCLC_A4_L82 line, VMLTs from five mice and the isotype negative control of the Tcam2 cell line representing the upper end of the range for isotype negative controls. Global 5mC levels were measured as Median Fluorescence Intensity (MFI) with error bars indicating Standard Error of the Mean (SEM). hiPSC_A4_L82 cell line exhibited very high 5mC levels consistent with its primed hESC state of somatic DNA hypermethylation pattern and previous studies (*35*). All VMLTs show nearly complete DNA demethylation for 5mC similar to that of isotype negative control. VP-MCC and VN-MCC cell lines also show extremely low 5mC levels similar to that of the Tcam-2 cell line, which is composed of transformed late-hPGCs and exhibit unique epigenetic signatures of late-hPGCs. Somewhat higher MFIs of TCam2 and VP-MCC cell lines than that of VMLTs most likely result from epigenetic drift of these cultured tumor cells rather than primary tumor cells (table S4).

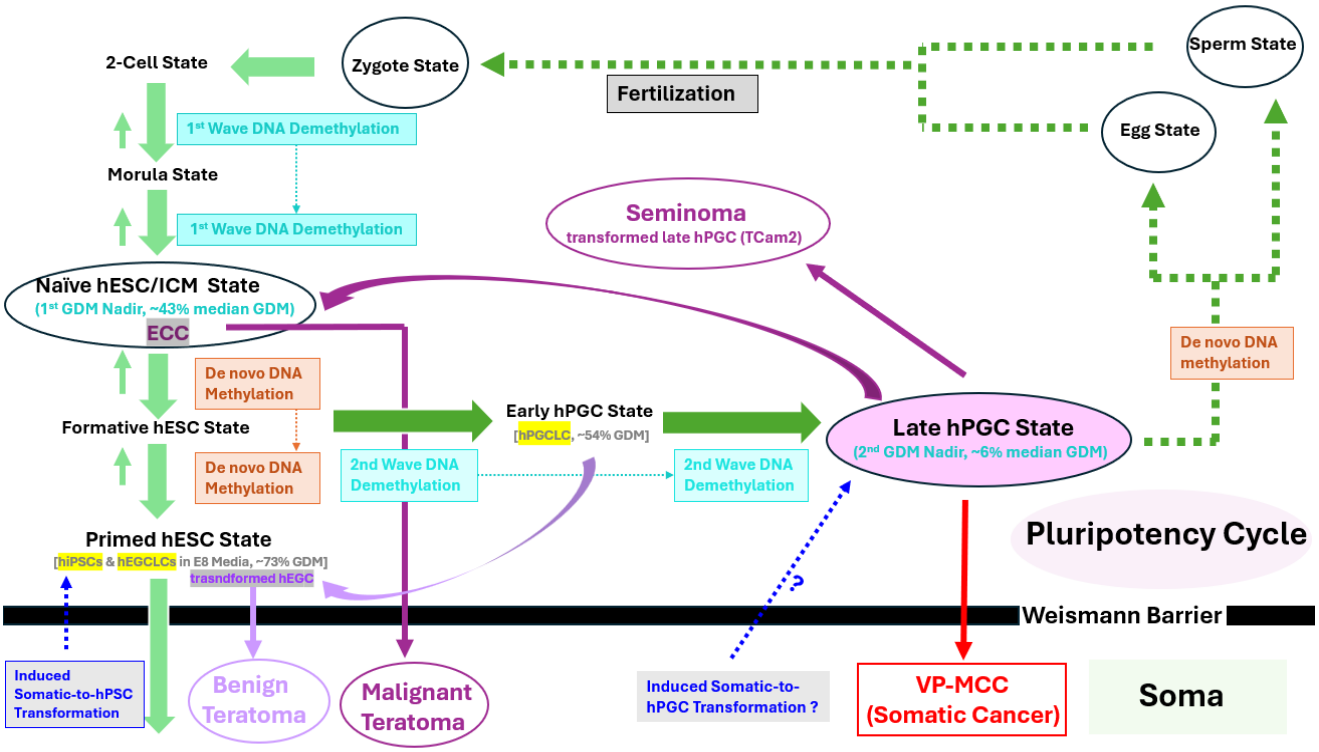

Fig. 5. Schematic of normal human embryonic and germline development in comparison to the carcinogenesis of somatic VP-MCC and germ cell tumors (GCTs). Light green arrows show normal early embryogenesis from fertilization to primed pluripotent hESCs before crossing the Weismann Barrier (WB) into somatic differentiation to form the soma. The intrinsic plasticity between the 2-cell stage and the primed hESC stage is reflected in the bidirectionality of the light green arrows (*1*). Normal human germline development (dark green arrows) begins at the specification of hPGCs from formative hESCs and ends with the formation of sperm or eggs, guided by an intrinsic program suppressing somatic fate. Notably, unipotent germline cells are the only human cell type capable of physiological conversion to pluripotency (parthenogenesis) without genetic manipulation, due to their latent pluripotency throughout germline development (*32*). Thus, the germline cycle may be considered a "pluripotency cycle" (*32*), consisting of both early embryogenesis (light green arrows) and germline development (dark green arrows), which are separated from the soma compartment by the Weismann Barrier (WB). Based on our mouse model, carcinogenesis of somatic VP-MCC (red straight arrow) also crosses the WB to the somatic compartment, similar to teratomas - a type of non-seminomatous GCT (NSGCT) (dark purple and light purple straight arrows) - and normal human embryogenesis (*1*). However, unlike teratomas or embryogenesis, VP-MCC tumorigenesis crosses the WB directly from the germline (late hPGCs) to the somatic side without a pluripotent intermediary, resulting in unilineage (neuroendocrine) somatic differentiation accompanied by developmental arrest. In contrast, tumorigenesis of malignant or benign teratomas more closely mirrors normal embryogenesis, requiring a totipotent (EC cell or ECC) or pluripotent intermediate to cross the WB and enable multilineage somatic differentiation. These totipotent or pluripotent stem cells of teratomas are believed to arise via parthenogenetic transformation of transformed hPGCs in vivo (dark purple and light purple curved arrows), rather than from fertilization-derived zygotes. Seminomas, another malignant GCT subtype, consist of transformed late hPGCs and therefore retain the epigenetic signatures characteristic of this stage (*1*). Two waves of global DNA demethylation (GDM) occur during development, each followed by de novo methylation. The first wave begins at the 2-cell stage and reaches its nadir at the naïve hESC/ICM stage, with partial erasure of GDM. The second, germline-specific wave occurs only during hPGC development, resulting in near-complete GDM erasure and reaching the lowest nadir at the late hPGC stage (*52*, *53*). The moderate global hypomethylation observed in two VN-vMCC cell lines similar to that of seminoma cell line TCam2 and VP-MCC lines suggests a possible somatic-to-hPGC transformation (dashed blue arrow with question mark), potentially triggered by somatic mutations or other mechanisms. This process may undergo indirect pathway through reprogramming of somatic cells into hiPSCs followed by specification into a hPGC-like state (dashed blue arrow). The three human primeval stem cell types used in our mouse model—hPGCLCs (modeling early hPGCs), hiPSCs (modeling primed hESCs), and hEGCLCs (modeling hEGCs)—are highlighted in yellow.