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ORIGINAL ARTICLE

Role of catecholamine synthases in the maintenance of cancer stem-like cells in malignant peripheral nerve sheath tumors

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Abstract

Malignant peripheral nerve sheath tumors (MPNSTs) are malignant tumors that are derived from Schwann cell lineage around peripheral nerves. As in many other cancer types, cancer stem cells (CSCs) have been identified in MPNSTs, and they are considered the cause of treatment resistance, recurrence, and metastasis. As an element defining the cancer stemness of MPNSTs, we previously reported a molecular mechanism by which exogenous adrenaline activates a core cancer stemness factor, YAP/TAZ, through β2 adrenoceptor (ADRB2). In this study, we found that MPNST cells express catecholamine synthases and that these enzymes are essential for maintaining cancer stemness, such as the ability to self-renew and maintain an undifferentiated state. Through gene knockdown and inhibition of these enzymes, we confirmed that catecholamines are indeed synthesized in MPNST cells. The results confirmed that catecholamine synthase knockdown in MPNST cells reduces the activity of YAP/TAZ. These data suggest that a mechanism of YAP/TAZ activation by de novo synthesized adrenaline, as well as exogenous adrenaline, may exist in the maintenance of cancer stemness of MPNST cells. This mechanism not only helps to understand the pathology of MPNST, but could also contribute to the development of therapeutic strategies for MPNST.

KEYWORDS
benserazide, cancer stem cell, catecholamine synthase, malignant peripheral nerve sheath tumor, Schwann cell, vesicular monoamine transporter

Abbreviations: ADRB2, β2 adrenoceptor; CSC, cancer stem-like cell; DBH, dopamine β-hydroxylase; DDC, DOPA decarboxylase; MPNST, malignant peripheral nerve sheath tumor; NGFR, nerve growth factor receptor; OS, overall survival; PNMT, phenylethanolamine N-methyltransferase; TAZ, transcriptional coactivator with PDZ-binding motif; TH, tyrosine hydroxylase; VMAT, vesicular monoamine transporter; YAP, yes-associated protein.

1 | INTRODUCTION

Malignant peripheral nerve sheath tumor is thought to be a malignant transformation of Schwann cell lineage around peripheral nerves.[1,2] Treatment outcomes for MPNST have not dramatically improved due to its high resistance to chemotherapy and radiation therapy.[3,4] As in many other cancer types, MPNSTs are known to have a highly stem-like cell population. In general, CSCs can be characterized by high self-renewal capacity, tumorigenic potential, ability to maintain an undifferentiated state, and drug resistance, and are considered to be the source of metastasis and recurrence.[5,6] Cancer stem-like cells are important in explaining tumor heterogeneity. Cancer stem-like cells are maintained by tumor microenvironments that consist of multiple components such as inflammation, hypoxia, vascular bed, and rigid matrix interactions. Tumor microenvironments also form tumor heterogeneity that reflects various differentiation states of CSCs.[7–9] We previously identified adrenaline as an in vitro humoral factor that expands the CSC population in MPNSTs.[10] We found that MPNSTs enhance their self-renewal capacity and undifferentiated state by activating cancer stem cell-associated factor YAP/TAZ, mainly through activation of ADRB2. In subsequent validation, we confirmed that adrenaline is the only catecholamine capable of expanding the CSC population of MPNSTs (Huang and Fujimura, 2023, unpubl. data).

Given the fact that tumor cells expressing ADRB2 in tumor tissues of MPNST patients coexpress CD133, an MPNST stem cell marker, and YAP/TAZ is activated in those cells,[10] that Schwann cells have the ability to dedifferentiate into Schwann precursor cells that generate chromaffin cells due to their plasticity,[11–13] and that the general blood adrenaline concentration is 0.1 nM, which seems incapable of increasing the stemness of the tumor,[14] we speculated that there could be other local sources of adrenaline besides blood adrenaline that contribute to the stemness of MPNST.

One possible source is the direct supply of adrenaline from the sympathetic nervous system. In some cancer types, sympathetic and parasympathetic infiltration around tumor tissue has been reported to contribute to cancer progression.[15,16] However, in our preliminary study, these findings are not the case in MPNSTs (Huang and Fujimura, 2023, unpubl. data). We next hypothesized that MPNST cells themselves synthesize adrenaline. This is because MPNSTs are malignant tumors originating from Schwann cell lineage, which are highly plastic and have been reported to possess dedifferentiation and transdifferentiation potential.[11] Schwann cell precursors have also been reported to be the origin of chromaffin cells in the adrenal medulla, which is a key area of biological catecholamine production.[12,13] Taking into account these previous reports, we hypothesized that Schwann cells may acquire the ability of catecholamine synthesis during their oncogenic transformation into MPNSTs. In this paper, we will examine whether the group of catecholamine synthesizing enzymes expressed in MPNST cells are essential for the maintenance of cancer stemness of MPNSTs. We will also examine whether inhibition of these enzymes might contribute to the improvement of therapeutic strategies against MPNSTs.

2 | MATERIALS AND METHODS

2.1 | Cell culture and reagents

The human MPNST cell line FMS-1 was kindly provided by Dr. Hakozaki (Department of Orthopedic Surgery, Fukushima Medical University),[17] HS-PSS and HS-Sch-2 were purchased from RIKEN BioResource Research Center (RCB2362 and RCB2230).[18] Immortalized mouse Schwann cell line IMS32 was purchased from Cosmo Bio.[19] The MPNST cell lines were maintained in RPMI-1640 (FUJIFILM Wako Chemicals) supplemented with 10% FBS (Nichirei Bioscience) and antibiotics (FUJIFILM Wako Chemicals). IMS32 was maintained in culture medium for Schwann cell line IMS32, including DMEM, serum, and antibiotics (Cosmo Bio).

2.2 | Lentivirus preparation and infection

For RNAi experiments, we prepared shRNA expressing lentivirus particles as previously described.[20] Briefly, 293FT cells (Thermo Fisher Scientific) were transfected with the pLKO.1-puro-shRNA expressing vector, psPAX2, and pMD2.G (Addgene) using TransIT-LT1 Transfection Reagent (TaKaRa Bio) and Opti-MEM (Thermo Fisher Scientific). The virus-containing medium was harvested 60 h after transfection. The following are the target sequences of RNAi used in this study: Human TH#1, GACGTACCAGTCAGTCTACTT; Human TH#2, GTTCGGGCTGTGTAAGCAGAA; Human DDC#1, GCTGG CCTGATTCCTTTCTTT; Human DDC#2, CGACGTTGAGAAGATAA TCAT; Human DBH#1, GCGGGTGCCGCTTAAACATTT; Human DBH#2, CTTCAACCGCGACGTACTGAA; Human PNMT#1, GAGGA CATCACCATGACAGAT; Human PNMT#2, GCACCCTCATCGACATT GGTT; Human VMAT1#1, GCGTACCTAGTGGAATAGCAT; Human VMAT1#2, GCTGGCTTTGTTATCATGTTT; Human VMAT2#1, GCTCTTCTGGGAATGATAATT; Human VMAT2#2, GGATCACA ACTGCCCTATTAA; and Control, CCTAAGGTTAAGTCGCCCTCG.

2.3 | Human tumor specimens

Human MPNST specimens, relevant information, and anonymized medical data were obtained from Okayama University Hospital, following approval by the ethics committee of the hospital (approval number: 2009-032). We used the same patient dataset as previously reported.[10] All the experiments involving human specimens were carried out in accordance with the Declaration of Helsinki. Informed consent was obtained in the form of an opt-out method on the website of the Okayama University Hospital (http://www.hsc.okayama-u.ac.jp/ethics/koukai/master/wp-content/uploads/2022/02/2009-032.pdf).

2.4 | Quantification and statistical analysis

The immunostaining signals were quantified using ImageJ software (NIH). Unpaired two-tailed *t*-test was used to assess the differences between the two groups. One-way ANOVA with Tukey's multiple comparisons post hoc test was used for the comparison of more than two groups. Log-rank test was used for survival analysis. Statistical significance was set at $p < 0.05$. The significance level was defined as ns (no significance), $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. All analyses were undertaken using EZR (Jichi Medical University)[21] and GraphPad Prism 9.5.1.733 (GraphPad Software).

For the other materials and methods, see Appendix S1, Figure S1, and Table S1.

3 | RESULTS

3.1 | Malignant peripheral nerve sheath tumor cells express catecholamine synthases

We first tested whether MPNST cells express catecholamine synthases. Catecholamine synthases include: TH, which synthesizes L-DOPA from L-tyrosine; DDC, which synthesizes dopamine from L-DOPA; DBH, which synthesizes noradrenaline from dopamine; and PNMT, which synthesizes adrenaline from noradrenaline (Figure 1A).[22] Western blot analysis confirmed that MPNST cell lines FMS-1, HS-PSS, and HS-Sch-2 express TH, DDC, DBH, and PNMT (Figure 1B). As expected, these enzymes were localized in the cytoplasm (Figures 1C and S2a). These data indicated that MPNST cells express a group of catecholamine synthases.

Immunofluorescence staining with Abs against noradrenaline and adrenaline was also performed to demonstrate that these cells contained catecholamines in the cells; in FMS-1 and HS-PSS, signals indicating noradrenaline and adrenaline were identified in the cytoplasm (Figure 1D, Figure S2b). The adrenaline signal was attenuated by RNAi of TH, DDC, DBH, and PNMT, which showed the presence of de novo catecholamines in the cells, and the signal was enhanced by the addition of adrenaline to the medium, indicating that these Abs correctly recognized adrenaline in the cells (Figure 1E,F). Furthermore, immunofluorescence staining with Abs against the catecholamine synthases, DDC and PNMT, and adrenaline in histopathological specimens from MPNST patients showed their expression in MPNST cells but not in the surrounding normal muscle tissue that was resected with the tumor (Figure 1G). These data indicated that MPNST cells synthesize endogenous catecholamines through their own catecholamine synthases.

3.2 | Catecholamine synthases are required for maintenance of cancer stemness of MPNST cells

Next, we investigated whether the expression levels of catecholamine synthases affected the clinical course of MPNST patients and examined the association between the expression levels of TH, DDC, DBH, and PNMT, and OS using a dataset of MPNST patients. Although there were no significant differences, we found that OS tended to be worse in patients with higher expression levels of DDC and PNMT (Figure 2A). To investigate whether differences in the protein expression levels of DDC and PNMT affected the clinical course, immunofluorescence staining was undertaken on patient specimens obtained at Okayama University Hospital. DOPA decarboxylase expression was associated with metastasis progression (Figure 2B). As the presence of cancer stem cells has been suggested as a source of poor patient prognosis,[5,6,23] we next examined whether the catecholamine synthase population, including DDC, was indeed associated with stemness in MPNST cells. RNA interference of TH, DDC, DBH, and PNMT was undertaken on FMS-1 and HS-PSS cells. A sphere formation assay was carried out to assess the self-renewal capacity, which revealed that knockdown of these enzymes significantly attenuated the self-renewal capacity (Figures 2C and S2c). Furthermore, immunofluorescence staining of MPNST cells with Abs against CD133 and NGFR, which are known cancer stem cell surface markers, revealed that both CD133 and NGFR signals were attenuated in cells in which these enzymes were knocked down (Figures 2D and S2d,e). Furthermore, the colony-forming capacity was significantly weakened in cells in which these enzymes were knocked down (Figures 2E and S2f). Xenograft experiments were undertaken in immunocompromised mice, and FMS-1 cells in which DDC was knocked down with RNAi did not produce palpable tumors (Figure 2F). These data indicate that a group of catecholamine synthases, including DDC, is essential for the maintenance of cancer stemness in MPNST cells. In addition, the fact that similar phenotypes were observed not only in the RNAi of DDC and PNMT, but also in TH and DBH, suggests that the cancer stemness of MPNST is maintained through canonical intrinsic catecholamine synthesis pathways.

3.3 | Group of catecholamine synthases is essential for maintenance of YAP/TAZ activity in MPNST

We have previously reported that YAP/TAZ, the Hippo transducer, is essential in the catecholamine-dependent cancer stemness maintenance mechanism in MPNST.[10] It has also been reported that YAP/TAZ plays a central role in the pathogenesis of MPNST.[24] Therefore, we investigated whether YAP/TAZ is involved in the mechanism of cancer stemness maintenance of MPNST by analyzing FMS-1 samples with RNAi of TH, DDC, DBH, and PNMT, which revealed that the YAP/TAZ protein was attenuated by the knockdown of these enzymes (Figure 3A–D). In addition, the expression of AXL and CYR61, the target genes of YAP/TAZ, and CD133, a stem cell surface marker of MPNST, was reduced accordingly (Figure 3A–D). The rate of nuclear translocation of YAP/TAZ was also attenuated by RNAi of these enzymes (Figure 3E). As TH is known to be the rate-limiting factor in the efficiency of catecholamine synthesis,[25] it is reasonable

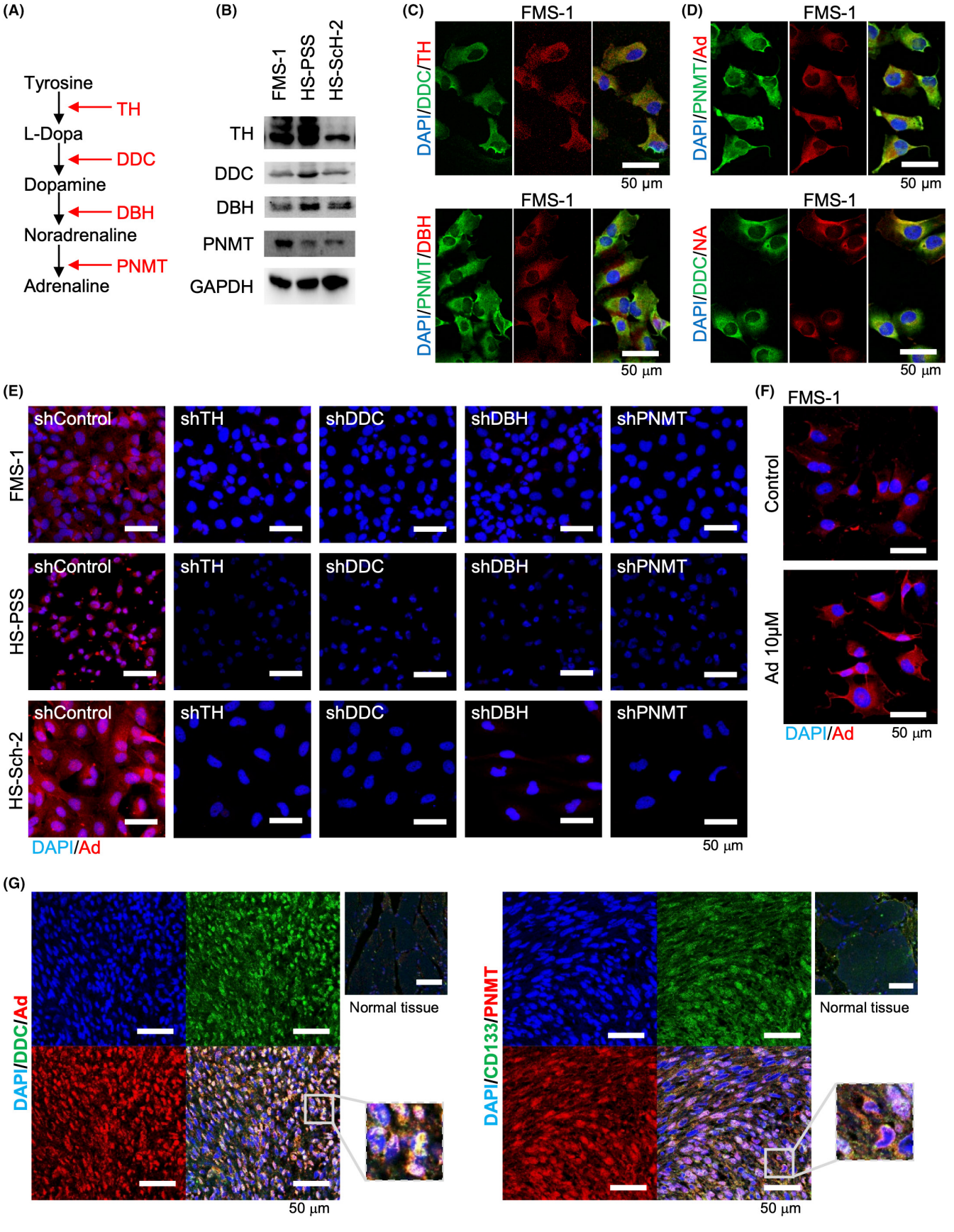

FIGURE 1 Malignant peripheral nerve sheath tumor (MPNST) cells express catecholamine synthases and these enzymes are involved in intracellular adrenaline synthesis. (A) Catecholamine synthetic pathway. (B) Catecholamine synthases were detected by western blot analysis in FMS-1, HS-PSS, and HS-Sch-2 cells. (C) Catecholamine synthases were detected in the cytoplasm by immunofluorescence staining in FMS-1 cells. Scale bar, 50 μm. (D) Adrenaline and noradrenaline were detected in the cytoplasm by immunofluorescence staining in FMS-1 cells. Scale bar, 50 μm. (E) Knockdown of catecholamine synthases attenuated the signals of cytoplasmic adrenaline in FMS-1, HS-PSS, and HS-Sch-2 cells. Scale bar, 50 μm. (F) Adrenaline signal is enhanced by the addition of adrenaline to the medium. Scale bar, 50 μm. (G) Immunofluorescence staining of MPNST patients' specimens. Catecholamine synthases (DOPA decarboxylase [DDC] and phenylethanolamine N-methyltransferase [PNMT]) and adrenaline were actually detected. Signals are stronger than those of normal muscle tissue. Scale bar, 50 μm. DBH, dopamine β-hydroxylase; TH, tyrosine hydroxylase.

to expect that the effect of RNAi on YAP/TAZ and stem cell markers would be stronger for TH than for PNMT. These data indicate that the catecholamine synthase group is essential for maintaining YAP/TAZ activity in MPNSTs.

3.4 | Inhibitors of the catecholamine synthesis pathway suppress cancer stemness of MPNST cells

Based on previous data, we considered catecholamine synthases to be promising therapeutic targets for MPNST. To inhibit endogenous catecholamine synthesis, we analyzed samples of MPNST cells treated with benserazide,[26] an inhibitor of DDC, one of the two components of the oral anti-Parkinson therapeutic combination (with the other component being L-DOPA), and nepicastat,[27] an inhibitor of DBH. Immunofluorescence staining confirmed that these inhibitors attenuated adrenaline signaling in the cells (Figure 4A). Treatment with benserazide and nepicastat reduced the self-renewal and colony-forming capacity of MPNST cells, signals of YAP/TAZ and its target genes *AXL* and *CTGF*, and CD133, a cancer stem cell surface marker (Figure 4B–D). The rate of nuclear translocation of YAP/TAZ was also attenuated by the DDC inhibitor benserazide (Figure 4E). FMS-1 cells were treated with the DDC inhibitor benserazide under different concentrations and cell proliferation was evaluated using MTS assay, which showed significant cell growth inhibitory activity at more than 10 μM (Figure 4F). To examine the cell-killing effect of benserazide, three MPNST cell lines (FMS-1, HS-PSS, and HS-Sch-2) and mouse Schwann cell line (IMS32) were cultured under different concentrations of benserazide for 72 h and evaluated using MTS assay; IC_{50} values were 15.0, 9.8, 10.1, and 86.3 μM, respectively (Figure 4G). Sphere formation assay was carried out with various concentrations of FMS-1 pretreated with 15 μM benserazide and without benserazide. This showed a significant decrease in the effective concentration of doxorubicin on the sphere-forming capacity of FMS-1 cells pretreated with benserazide (Figure 4H). Although it has been previously reported that benserazide exerts its cell-killing effect in various cancer cell lines at concentrations above 100 μM by inhibiting HK2,[28] we have now determined that benserazide exerts sufficient cell growth inhibition and cell-killing effects at lower concentrations in MPNST cells. These data suggest that catecholamine synthesis in MPNST cells is important for YAP/TAZ-mediated maintenance of cancer stemness.

3.5 | Intracellular vesicles, important sites for synthesis of noradrenaline and adrenaline, are also required for maintenance of cancer stemness of MPNSTs

Intracellular vesicles are believed to be important sites for the synthesis of noradrenaline and adrenaline.[29–31] For example, noradrenaline is synthesized by DBH when dopamine is taken up into vesicles through VMAT. In MPNST cells, the expression of both VMAT1 and VMAT2 was confirmed (Figure 5A). The immunofluorescence staining of FMS-1 showed their localization in cytoplasm (Figure 5B). Vesicular monoamine transporter RNAi in FMS-1 and HS-PSS cells showed reduced self-renewal capacity (Figure 5C). We validated the effect of reserpine,[32] a VMAT inhibitor, and found that reserpine significantly attenuated the self-renewal and colony-forming capacities of FMS-1 cells (Figure 5D,E). Reserpine treatment also suppressed the ability to maintain an undifferentiated state, activation of YAP/TAZ, and its target genes *AXL* and *CTGF* (Figure 5F,G). These data suggested that the incorporation of monoamines into vesicles, the site of catecholamine synthesis, might contribute to the maintenance of MPNST cell stemness.

4 | DISCUSSION

Based on the fact that MPNSTs are malignant tumors derived from the Schwann cell lineage, Schwann cells are reported to have high plasticity, and Schwann cell precursors differentiate into chromaffin cells, we tested the hypothesis that MPNSTs express a group of catecholamine synthases. As predicted, MPNST cells expressed catecholamine synthases (Figure 1B,C). Immunofluorescence staining with specific Abs against noradrenaline and adrenaline revealed that MPNST cells synthesized catecholamines (Figure 1D,E). Importantly, these enzymes are essential for maintaining cancer stemness in MPNSTs (Figure 2). We identified catecholamine synthases as essential for the maintenance of YAP/TAZ activity, which is a core factor for cancer stemness (Figure 3). We previously reported that exogenous adrenaline enhances the stemness of MPNSTs by activating YAP/TAZ through ADRB2. The pathway discovered in this study indicates that endogenous adrenaline, which is synthesized de novo in MPNST cells, maintains cancer stemness through YAP/TAZ. Whether this molecular mechanism is specific to MPNSTs derived from Schwann cells or

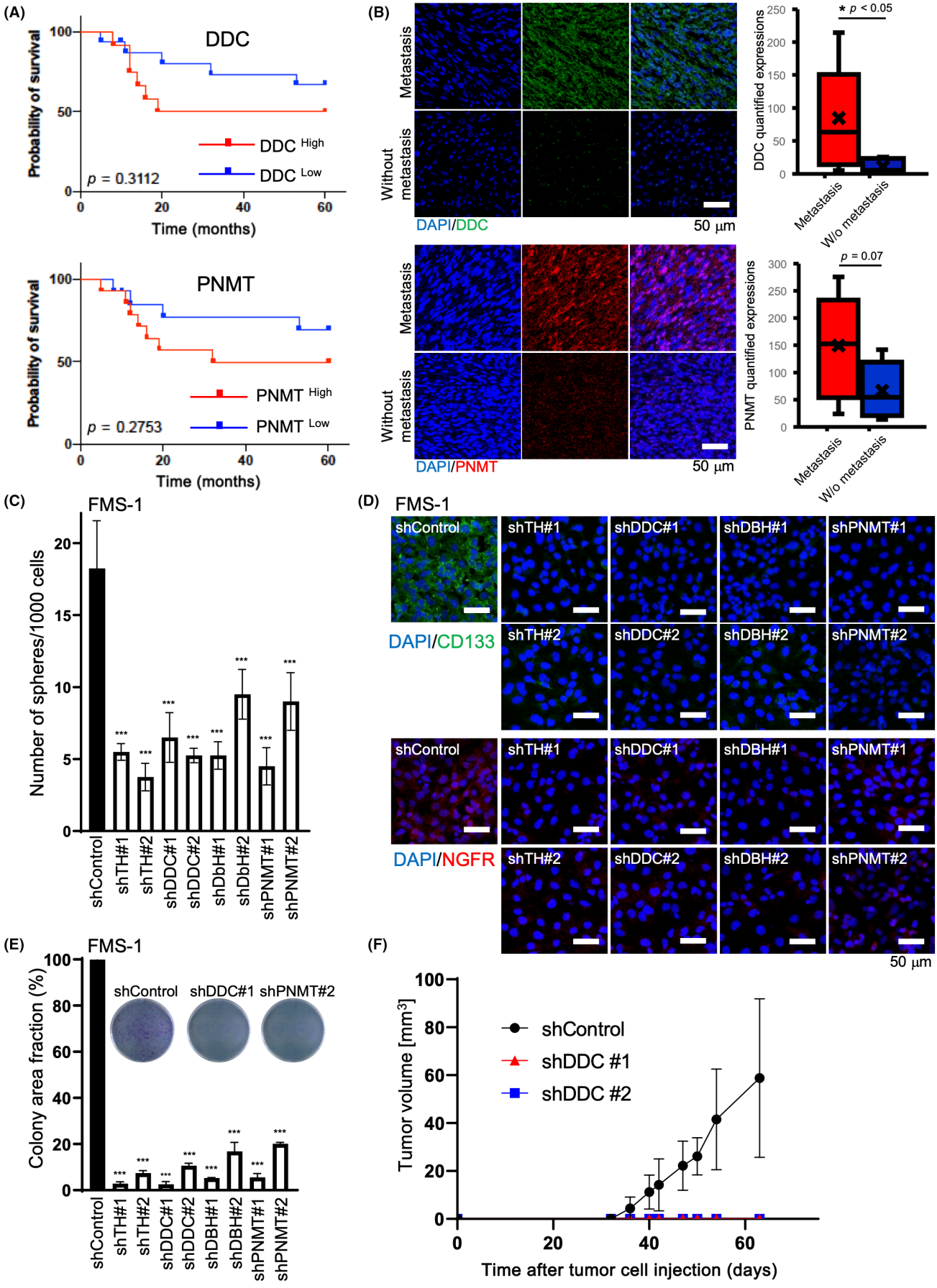

FIGURE 2 Group of catecholamine synthases is required for the maintenance of cancer stemness of malignant peripheral nerve sheath tumor (MPNST) cells. (A) Although not statistically significant, higher signals of DOPA decarboxylase (DDC) and phenylethanolamine N-methyltransferase (PNMT) tend to indicate worse prognosis of MPNST patients ($p=0.311$, $p=0.275$). (B) Immunofluorescence staining analysis of MPNST patients' specimens. DDC signal was significantly stronger in specimens from patients with metastasis (upper) than in those without metastasis (lower). PNMT signal was not significantly different between specimens from patients with metastasis (upper) and without metastasis (lower). (C) Knockdown of catecholamine synthases inhibited the self-renewal capacity of FMS-1 cells ($n=4$, error bars indicate mean ± SD). (D) Knockdown of catecholamine synthases attenuated the signals of CD133 and nerve growth factor receptor (NGFR) in FMS-1 cells. Scale bar, 50 μm. (E) Knockdown of catecholamine synthases inhibited the colony-forming capacity of FMS-1 cells ($n=3$, error bars indicate mean ± SD). (F) Knockdown of DDC inhibited the tumorigenesis of palpable tumor from FMS-1 in xenograft mouse ($n=10$, error bars indicate mean ± SD). $^{*}p < 0.05$, $^{***}p < 0.001$.

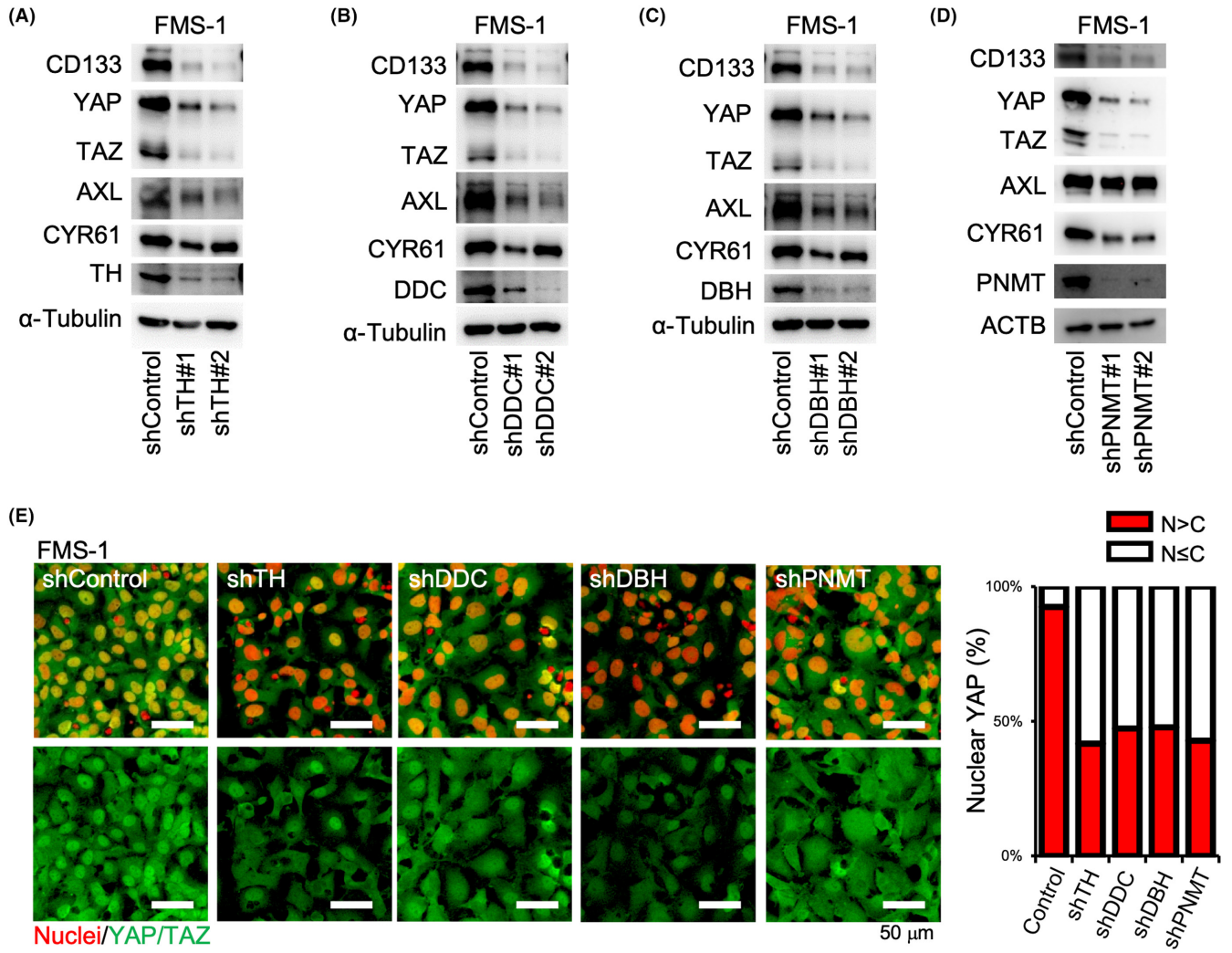

FIGURE 3 Catecholamine synthesizing enzymes are essential for the maintenance of YAP/TAZ activity in malignant peripheral nerve sheath tumor. (A–D) Tyrosine hydroxylase (TH), DOPA decarboxylase (DDC), dopamine β-hydroxylase (DBH), and phenylethanolamine N-methyltransferase (PNMT) knockdown attenuated YAP/TAZ, a transcriptional coactivator, its target genes, and stem cell marker, CD133. (E) Immunofluorescence analysis showed that knockdown of catecholamine synthases inhibited the nuclear accumulation of YAP/YAZ. The graph indicates the percentage of the cells with nuclear YAP/TAZ signal. Scale bar, 50 μm. Nuclei are indicated in pseudo-color. C, cytoplasmic localization; N, nuclear localization.

is observed in other common tumors, such as breast and lung cancers, remains to be verified.

In this study, we showed that inhibitors of the catecholamine synthesis pathway suppressed the stemness of MPNSTs. Among the inhibitors of enzymes involved in catecholamine synthesis, benserazide, which is used as an adjunct drug in L-DOPA substitution therapy for Parkinson's disease, is thought to increase the stability of L-DOPA by inhibiting DDC in the peripheral body tissues, resulting in increased L-DOPA translocation into the brain. In meta-analyses, the risk of carcinogenesis in patients with Parkinson's

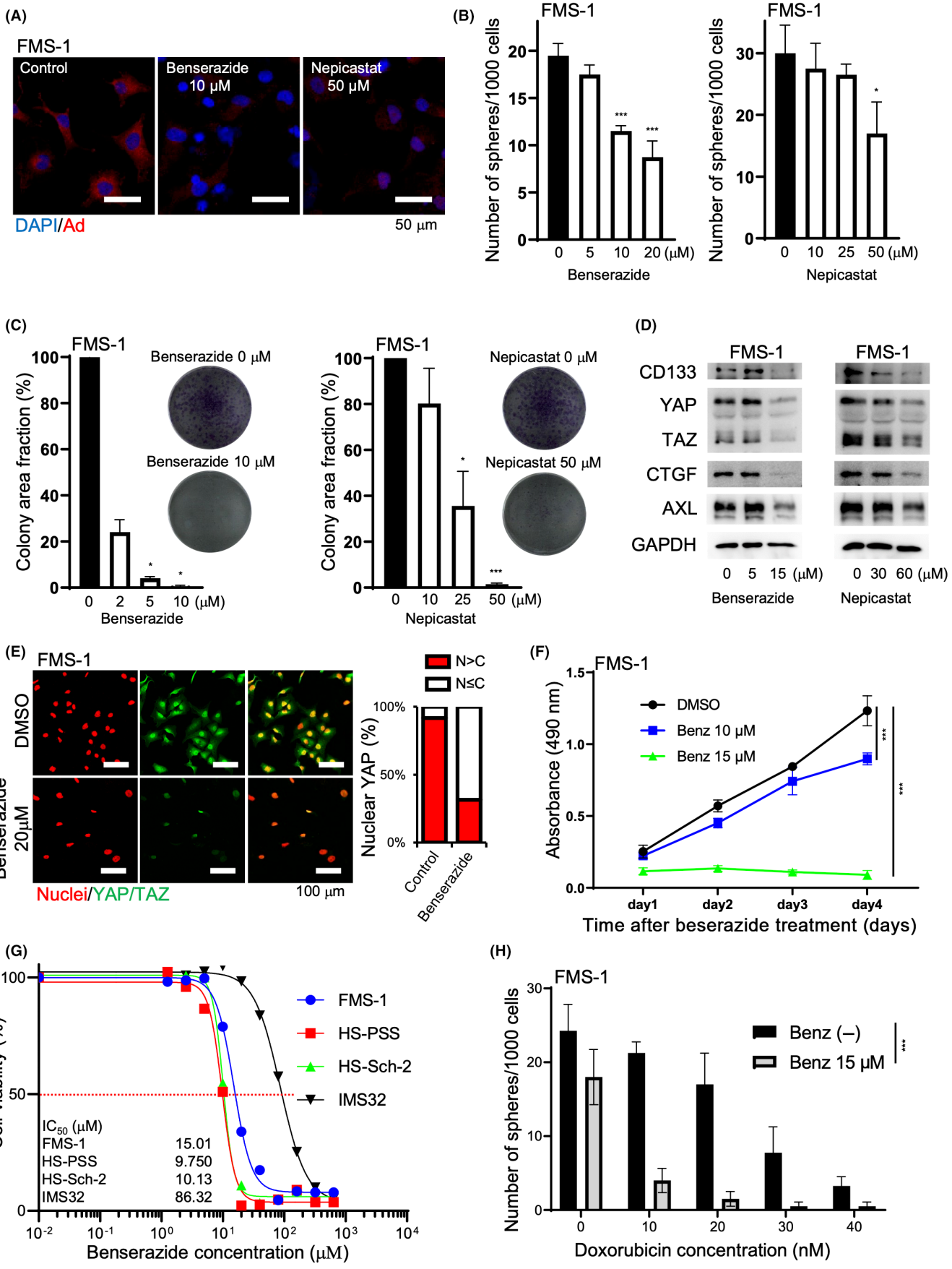

FIGURE 4 Inhibitors of catecholamine synthases suppress cancer stem cell-like characteristics of malignant peripheral nerve sheath tumor cells in vitro. (A) Benserazide and nepicastat attenuated the cytoplasmic signals of adrenaline in FMS-1 cells. Scale bar, 50 μm. (B, C) Benserazide and nepicastat significantly reduced the self-renewal and colony-forming capacity of FMS-1 cells in a concentration-dependent manner ($n = 4$, error bars indicate mean ± SD). (D) Benserazide and nepicastat attenuated YAP/TAZ, a transcriptional coactivator, its target genes, and stem cell marker, CD133. (E) Immunofluorescence staining analysis showed that benserazide inhibited the nuclear accumulation of YAP/YAZ. The graph indicates the percentage of cells with nuclear YAP/TAZ signal. Scale bar, 100 μm. Nuclei are indicated in pseudo-color. (F) Cell proliferation assay shows significant reduction of cell proliferation induced by benserazide (Benz). The reduction is concentration-dependent. (G) Sigmoid curves of FMS-1, HS-PSS, HS-Sch-2, and IMS32 cells against benserazide. IC_{50} values (μM) against benserazide: FMS-1, 15.01; HS-PSS, 9.75; HS-Sch-2, 10.13; IMS32, 86.32. (H) Pretreatment with benserazide (15 μM) had a synergistic effect with doxorubicin in the reduction of sphere formation capacity. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. C, cytoplasmic localization; N, nuclear localization.

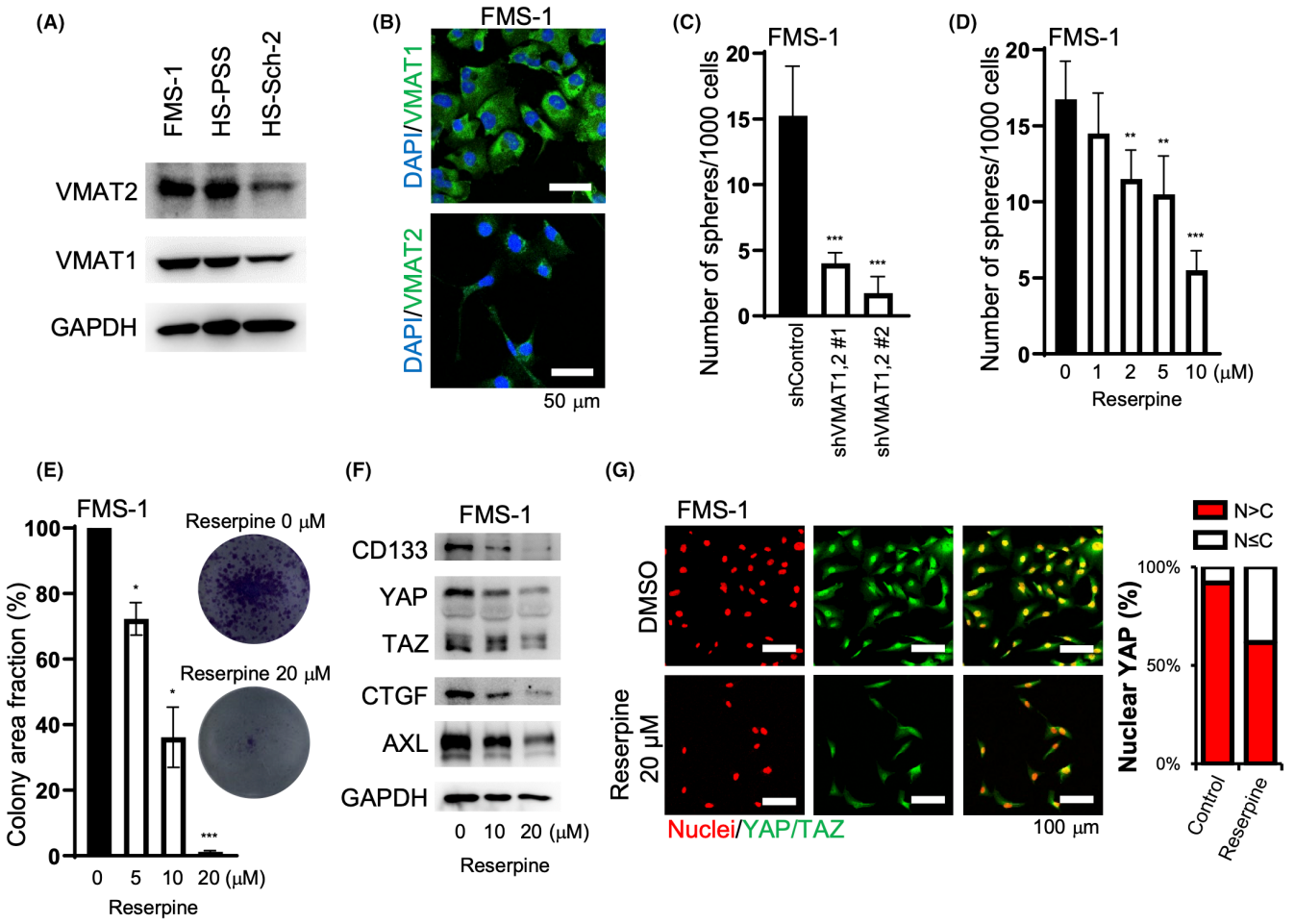

FIGURE 5 Inhibition of vesicular monoamine transporter (VMAT) also suppresses cancer stem cell-like characteristics of malignant peripheral nerve sheath tumor (MPNST) cells in vitro. (A) Expression of VMAT1 and VMAT2 was observed in each of the three MPNST cell lines. (B) VMAT1 and VMAT2 were detected in the cytoplasm using immunofluorescence staining in FMS-1 cells. Expression of VMAT2 is lower than that of VMAT1. Scale bar, 100 μm. (C) Knockdown of VMAT1 and VMAT2 inhibited the self-renewal capacity of FMS-1 ($n = 4$, error bars indicate mean ± SD). (D, E) Reserpine significantly reduced the self-renewal and colony-forming capacity of FMS-1 in a concentration-dependent manner ($n = 4$ and 3, error bars indicate mean ± SD). (F) Reserpine attenuated expression levels of YAP/TAZ, a transcriptional coactivator, its target genes, and stem cell surface marker, CD133. (G) Immunofluorescence staining analysis showed that reserpine inhibited the nuclear accumulation of YAP/YAZ. Graph indicates the percentage of the cells with nuclear YAP/TAZ signal. Scale bar, 100 μm. Nuclei are indicated in pseudo-color. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. C, cytoplasmic localization; N, nuclear localization.

disease tended to be lower than that in other populations.[33,34] These statistical data may provide partial support for the anticancer effect of benserazide. Previous studies have reported that benserazide has anticancer activity against various cancers.[28,35] These reports suggest that benserazide may exert its anticancer effect by inhibiting enzymes other than DDC; however, since the concentration

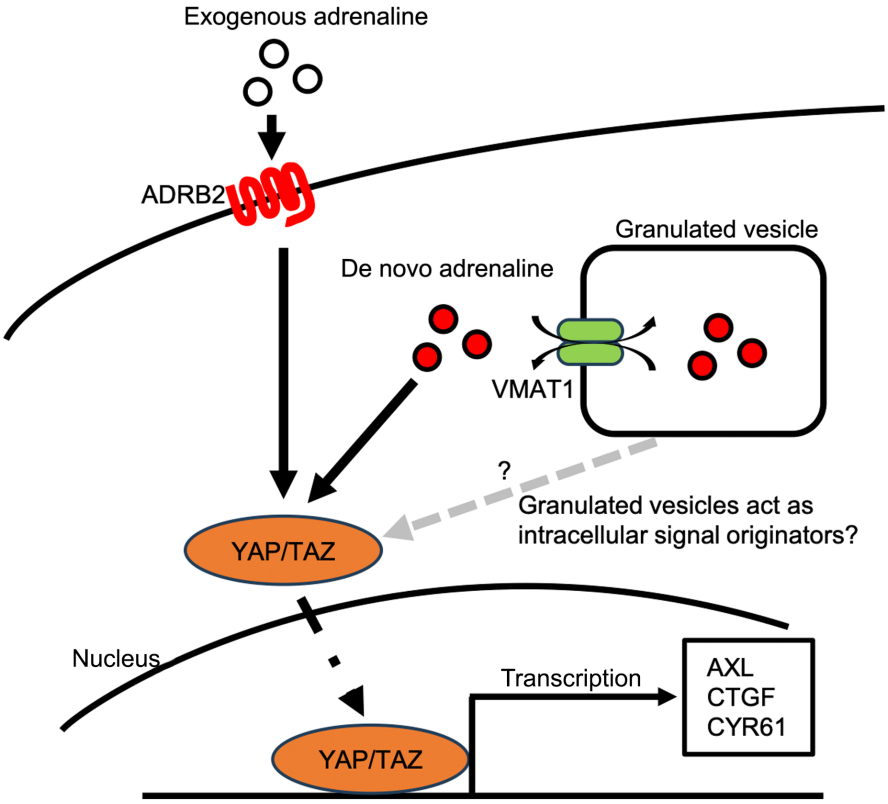

FIGURE 6 Summary of the results in this study. In the previously reported pathway, exogenous adrenaline activated YAP/TAZ through β2 adrenoceptor (ADRB2) to maintain cancer stem-like cells, whereas in this study, de novo synthesized adrenaline is involved in the maintenance of cancer stem-like cells. However, the style of signaling has not been clarified. VMAT, vesicular monoamine transporter.

of benserazide used in these studies was very high (approximately 100 μM), the possibility of nonspecific effects should be considered. This study showed that the difference in IC_{50} values between each tumor cell line and Schwann cell line may indicate the safety of benserazide for Schwann cell lineage, and that benserazide may exert synergism with doxorubicin, one of the most efficacious agents routinely used in the treatment of soft tissue sarcomas.[36] However, there are limitations in the safety of benserazide. In this study, we used only Schwann cell line for normal cell control. There have been several published works on the safety of benserazide. It has been reported that there are no appreciable effects of benserazide on the cardiovascular, renal, gastrointestinal, or central nervous systems in the usually independently administered doses.[37] It was also reported that benserazide at 300 and 600 mg/kg suppressed cancer growth in tumor-bearing mice and no toxicity shown.[28] Taking these findings into consideration, further studies are needed to clarify the safety of benserazide. Reserpine has also been reported to show anticancer activity.[38] Although no clear anticancer activity has been reported for nepicastat, basic research is being undertaken on it as a therapeutic target for psychiatric disorders, such as posttraumatic stress disorder and cocaine dependence.[27,39] Future studies are needed to determine whether these agents show anticancer activity in a xenograft model of MPNST cells.

In the present study, VMAT, which is also required for the de novo adrenaline synthesis pathway, was detected in MPNST. These results indicate that not only the group of catecholamine synthases, but also the catecholamine synthesis pathway itself is involved in the maintenance of cancer stemness of MPNST and can be a potential therapeutic target for MPNST.

In this study, we report that de novo catecholamine synthesis in MPNST cells is essential for maintaining CSCs through YAP/TAZ activation. Whether this de novo adrenaline acts in an autocrine or paracrine manner or is completed only within the cells remains to be determined (Figure 6). Self-renewal capacity, which was attenuated by TH RNAi, was not sufficiently rescued by the addition of exogenous adrenaline (Huang and Fujimura, 2023, unpubl. data). This suggests that de novo-synthesized adrenaline could be the origin of signaling pathways in autologous cells, and future studies on the signaling mechanism of endogenous catecholamines are warranted.

AUTHOR CONTRIBUTIONS

Haruyoshi Katayama: Data curation; formal analysis; funding acquisition; investigation; visualization; writing – original draft. **Atsushi Fujimura:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; project administration; resources; supervision; writing – original draft; writing – review and editing. **Rongsheng Huang:** Formal analysis; investigation; methodology. **Yusuke Otani:** Formal analysis; investigation; methodology. **Takuto Itano:** Formal analysis; investigation; methodology. **Tomohiro Fujiwara:** Writing – review and editing. **Toshiyuki Kunisada:** Writing – review and editing. **Eiji Nakata:** Conceptualization; project administration; supervision; writing – review and editing. **Toshifumi Ozaki:** Project administration; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

Approval of the research protocol by an institutional review board: Human MPNST specimens, relevant information, and anonymized medical data were obtained from the Okayama University Hospital following approval by the ethical committee of the hospital (approval number: 2009-032).

Informed consent: Informed consent was obtained through the website of the Okayama University Hospital (http://www.hsc.okayama-u.ac.jp/ethics/koukai/master/wp-content/uploads/2022/02/2009-032.pdf) with an opt-out option.

Registry and the registration no. of the study/trial: N/A.

Animal studies: Animal experiments were performed with permission from and in accordance with all guidelines put forth by the committees of the Okayama University (approval number: OKU-2021331 for recombinant DNA experiments on transgenic mice).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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