Neurologia medico-chirurgica Advance Publication Date: August 6, 2024

Special Topic doi: 10.2176/jns-nmc.2024-0078

Translational Research Platform for Malignant Central Nervous System Tumors

Kensuke Tateishi[1,2,3]

[1]*Department of Neurosurgery, Graduate School of Medicine, Yokohama City University, Yokohama, Kanagawa, Japan*
[2]*Laboratory of Biopharmaceutical and Regenerative Science, Graduate School of Medical Science, Yokohama City University, Yokohama, Kanagawa, Japan*
[3]*Neurosurgical-Oncology Laboratory, Yokohama City University, Yokohama, Kanagawa, Japan*

Abstract

Some central nervous system (CNS) malignancies are highly aggressive and urgently need innovative treatment strategies to improve prognosis. A significant concern for therapeutic development is the time-consuming nature of developing treatments for CNS tumors. Therefore, a rapid and efficient translational approach is needed to address this problem. Translational and reverse translational research aims to bridge the gap between laboratory data and clinical applications and has been developed in the field of neuro-oncology. This study presents our translational platform systems for malignant CNS tumors, which combine an intraoperative integrated diagnostic system and comprehensive *in vitro* and *in vivo* assay systems. These laboratory systems may contribute to a better understanding of tumor biology and the development of novel therapeutic strategies for the poor prognosis of CNS tumors.

Keywords: central nervous system tumors, translational research, precision medicine

Introduction

Central nervous system (CNS) malignancies are uncommon; however, some diseases have a poor prognosis. For instance, glioblastoma, IDH wild-type (GBM), is one of the major malignant brain tumors and remains a fatal disease, despite the establishment of standard therapy.[1)] These tumors have unmet medical needs and innovative treatment strategies are required to improve treatment results. A significant concern for therapeutic development is the time-consuming nature of developing treatments for CNS tumors. For example, conventional clinical approaches initiate exploratory research, followed by preclinical research and clinical trials, after which therapeutic drugs are created. It took almost 20 years to develop temozolomide (TMZ) as a standard chemotherapeutic agent for GBM.[2,3)] Therefore, a rapid and efficient translational approach is required.

In the past decade, translational research (TR) and reverse TR approaches have been developed in the field of medicine.[4)] Research on TR and reverse TR aims to bridge the gap between laboratory data and clinical applications. Preclinical research can be promoted and advanced by combining TR and reverse TR, leading to the acceleration of its clinical applications. Indeed, by making full use of the TR and reverse TR approaches, it is becoming possible to accelerate decision-making, improving the efficiency of drug discovery by promoting disease understanding, and accelerating clinical application. Recent comprehensive studies have identified genomic characteristics[5-11)] that provide molecular-targeted therapy for selected tumors.[12-14)] In this study, we present our TR platform systems for malignant CNS tumors and discuss how to develop clinical and research activities in the field of neuro-oncology.

Materials and Methods

Institutional review board

Patients with CNS tumors who underwent surgery at Yokohama City University (YCU) Hospital or its affiliated

Received April 8, 2024; Accepted June 12, 2024

hospitals were enrolled in this study. All studies were conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Yokohama City University (YCU, Yokohama, Japan; IRB numbers: A171130006 and B190700012). Written informed consent was obtained from all the patients. All animal experiments were approved by the Institutional Animal Care and Use Committee of the YCU (FA22-011). Detailed preclinical research information, such as histopathological and biochemical analyses, genomic and epigenomic analyses, and animal experiments, has been previously described.[15-19)]

Creation of central nervous system tumorsphere lines and cell culture

All tumor and blood samples were collected at YCU Hospital. To create CNS tumorsphere lines, fresh tumor specimens were obtained from surgery, dissected, and enzymatically dissociated using 0.1% of trypsin and DNase. Glioma and CNS lymphoma (CNSL) tumorspheres were cultured in serum-free EF20 medium, which is composed of neurobasal medium (Gibco) with 1x B27 supplement (Gibco), 0.25× N_2 (Gibco), L-glutamine (3 mM, Gibco), 1x Antibiotic-Antimycotic (Gibco), 20 ng/mL recombinant human epidermal growth factor (EGF, R&D), and 20 ng/mL recombinant human fibroblast growth factor (FGF)-basic (Alomone Labs). To culture ependymoma and meningioma cells, Meningeal Cell Medium (ScienCell) with fetal bovine serum (FBS), meningeal growth supplement, and antibiotic solution (ScienCell) was used. To create other CNS tumor cell lines, Dulbecco's Modified Eagle's Medium or RPMI-1640 with 10% of FBS and 1x Antibiotic-Antimycotic was used. All tumorsphere lines were cryopreserved at less than passage five to use for *in vitro* experiments and genomic analysis.

Cell viability analysis

To assess cell viability, tumorspheres were dissociated into single cells and seeded into 96-well plates at 3,000-8,000 cells/well. After 6-24 h, chemical inhibitors were serially diluted and added to the wells. Cell viability was measured using the CellTiter-Glo assay (Promega) on Day 3.

Orthotopic xenograft model

Tumorsphere cells (1-2 × 10^5) were orthotopically implanted into the right striatum of 4-9-week-old female severe combined immunodeficiency disease beige mice (Charles River, Yokohama, Japan). The mice were monitored at least three times a week and euthanized when neurological deficits or general conditions met the euthanasia criteria. Brains were harvested for pathological and genomic studies, and acutely dissociated tumor cells were cryopreserved with Bambankar (GCLTEC), cultured for *in vitro* experiments, or repeatedly implanted into mouse brains to propagate tumor cells *in vivo*.

Intraoperative integrated diagnostic system (*i*-ID)

After tumor sampling, the tumor specimens were divided by laboratory staff for frozen section (FS) analysis, genomic analyses, cell culture, cell viability assays, and animal experiments. When hematoxylin and eosin staining suggested malignant CNS tumors, rapid immunohistochemistry (R-IHC) with GFAP and CD20 antibodies was performed for differential diagnosis between gliomas and CNSL. If CD20 R-IHC was positive in the tumor cells, further sampling was terminated. In contrast, glioma was suspected if the pathological features did not indicate lymphomas or if GFAP-positive tumor cells were present. In such cases, neurosurgeons were informed of the tumor phenotype by pathologists and tumor resection was continued. Intraoperative genomic analysis was performed using rapidly extracted DNA. If a glioma was histopathologically suspected, genotyping for $IDH1^{R132H}$, $IDH2^{R172K}$, $TERT^{C228T\ and\ C250T}$ single nucleotide variants (SNVs), and *CDKN 2A* copy number alteration (CNA) was routinely performed, whereas $BRAF^{V600E}$ and $H3F3A^{K27M}$ SNVs were assessed in selected cases. After merging the histopathological and molecular information, *i*-ID was determined and *i*-ID information was immediately shared with physicians.

Results

Translational research platforms for malignant CNS tumors

Figure 1 shows an overview of the CNS malignant tumor research platform in our laboratory. Histopathological analysis was routinely performed using a clinical approach. DNA and RNA were extracted from tumor specimens, and genetic and epigenetic abnormalities were evaluated using a multi-omics approach (reverse TR approach). The tumor and blood samples were stored as biobank samples for future research. Primary cultured cells were generated for drug sensitivity assays and to establish preclinical models (TR approach). Our research aimed to elucidate tumor biology and responsiveness to chemotherapeutic agents and develop individualized therapies for patients with malignant CNS tumors. To complete these missions, it is desirable to use reproducible *in vitro* and *in vivo* models. Patient-derived xenografts (PDX), in which tumors are xenotopically transplanted into immunodeficient animals, are highly reproducible models that mimic patient characteristics, including the phenotype and genotype.[16,18-20)] Therefore, these models can be used to uncover gene-specific tumor biology and develop gene-specific target therapies. Based on this, our laboratory has attempted to establish PDX mouse models and PDX-derived cell lines (PDCs). Once the condition of the mice became lethal, the xenografts were extracted and used for experiments. Histopathological assessment was performed to evaluate phenotypic characteristics. Genetic and epigenetic profiling were performed to assess the consistency of patient profiling.

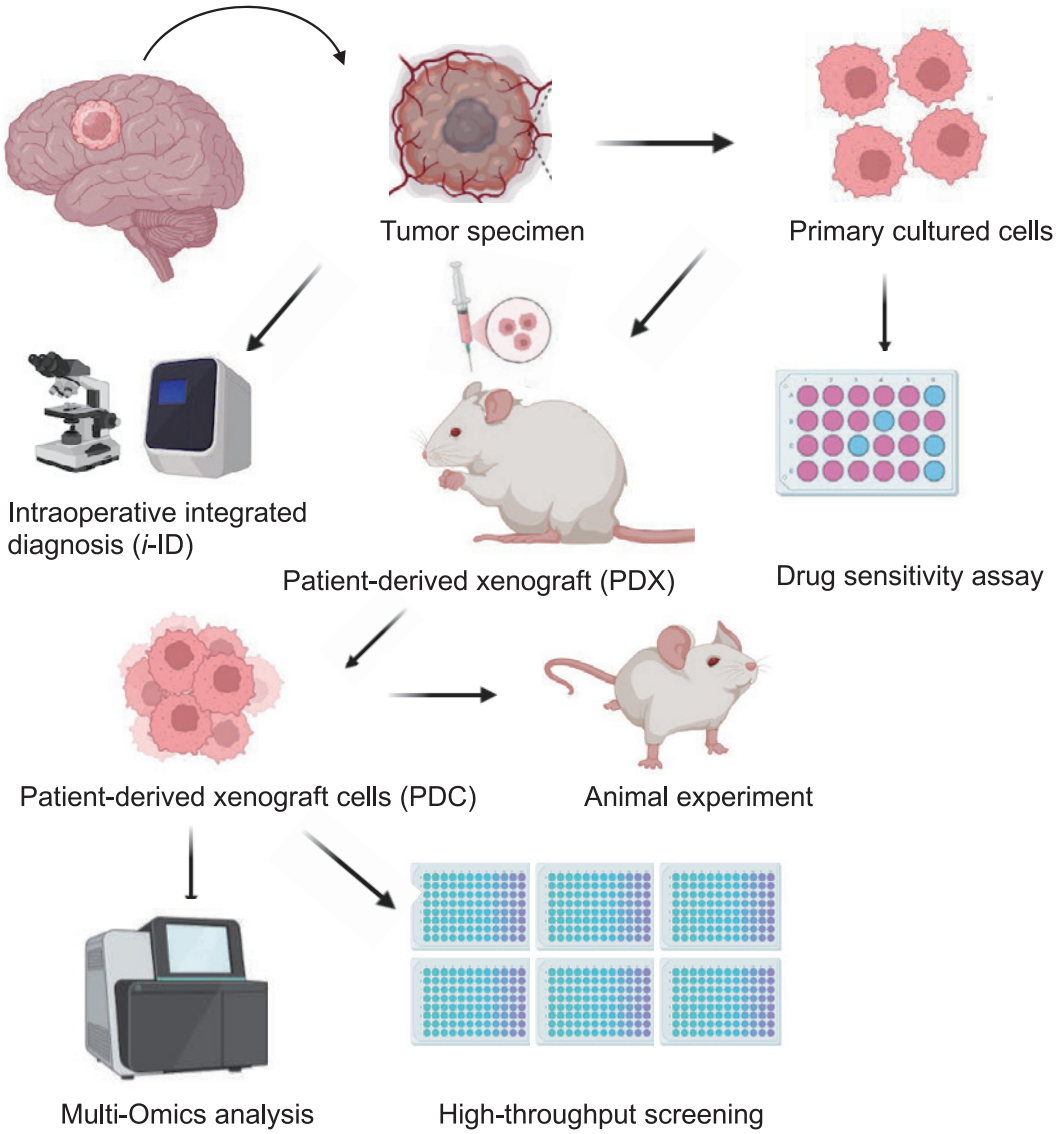


Fig. 1 Translational research platform at our laboratory (Neurosurgical-Oncology lab, YCU).

We also analyzed these genomic and epigenetic alterations to confirm whether they were critical genetic and epigenetic events that promoted xenograft formation using multiple approaches (TR approach). Drug screening assays, such as high-throughput drug screening and *in vitro* experiments, were performed using cultured PDCs. PDCs were also repeatedly implanted into mouse brains for *in vivo* propagation. By merging these data, we attempted to identify therapeutic targets and develop novel treatment strategies using PDX-derived tumor cells (TR approach). In addition, as a result of these approaches, we collected malignant CNS tumor cells from more than 450 cases, such as 327 gliomas, 63 CNSL, and 34 meningiomas. We established multiple PDX models, such as 146 glioma PDX and 43 CNSL-PDX, to date. PDX formation rates were 56.2% (146/260) and 72.5% (43/63) in gliomas and CSNL, respectively. We currently use these lines in clinical and research activities.

Intraoperative Integrated Diagnostic System for CNS Malignant Tumors

Recently, we developed an original intraoperative integrated diagnostic system (*i*-ID) that provides a reliable integrated diagnosis of adult malignant CNS tumors.[15] By combining FS assessment, intraoperative immunohistochemistry, and qPCR-based genotyping assays, such as *IDH1/2*, *TERT* promoter mutation, and *CDKN2A* CNA, we prospectively assessed the potential for CNS malignant tumors during surgery. We found that the qPCR-based genotyping assay detected mutant alleles in more than 1%. Assessment of *IDH1/2*, *TERT*, *H3F3A*^{K27M}, *BRAF*^{V600E} single nucleotide variants (SNVs), and *CDKN2A* CNA with *i*-ID and permanent genomic analysis was concordant in 100%,

100%, 100%, 100%, and 96.4%, respectively. We found that *i*-ID system provided 97.0% (98/101) accuracy in classifying CNS malignant tumors according to the World Health Organization (WHO) 2021 criteria in a prospective cohort. Conversely, *i*-ID were mismatched with a permanent integrated diagnosis in three cases. In one case, *H3F3A*^{G34V} mutation was identified, and the diagnosis was changed from glioblastoma, IDH wild-type (*i*-ID), to diffuse hemispheric glioma, H3 G34-mutant. Moreover, we encountered two cases with a low tumor content in tissue samples, in which genomic assessment or R-IHC was misinterpreted. This indicates a potential limitation of the current *i*-ID system in cases with a low tumor content ratio or low variant allele frequency. Nonetheless, these findings demonstrate that *i*-ID system modernizes intraoperative histopathological analysis. In addition, this system can be used as a platform for multidisciplinary interventions during surgery.[15,21]

Illustrative Cases

Case 1

A 45-year-old woman (YMG265) visited the clinic with a mild headache. Magnetic resonance imaging (MRI) detected an abnormal signal intensity in the right occipital lobe. She was then transferred to our hospital for surgery. FS assessment suggested a CNS WHO grade 3 glioma, but the histopathological differentiation was not possible. We used the *i*-ID system to assess genomic alterations and found *IDH1*^{R132H} and *TERT*^{C228T} mutations in the tumor (reverse TR approach). Thus, we primarily diagnosed this tumor as an oligodendroglioma, IDH mutant, and CNS WHO grade 3 during surgery. Based on this information, we conducted maximal tumor resection rather than supramaximal resection because a recent large study did not provide survival evidence for supramaximal resection in patients with oligodendroglioma.[22] Postoperative examination revealed the presence of *IDH1*^{R132H} and *TERT*^{C228T} mutations and a chromosome 1p/19q codeletion, and the integrated diagnosis was determined as oligodendroglioma, IDH mutant, and 1p/19q-codeleted, CNS WHO grade 3. We also performed drug screening to determine the appropriate adjuvant treatment regimen. Accordingly, we found that the primary cultured cells were relatively sensitive to TMZ and subsequently the patient received radiotherapy with TMZ chemotherapy (TR approach, Fig. 2A-E). The patient has remained in complete remission for 12 months without any morbidity. Similar to our previous study,[19] the PDX model could not be established from this tumor, together with the favorable clinical course of the patient.

Case 2

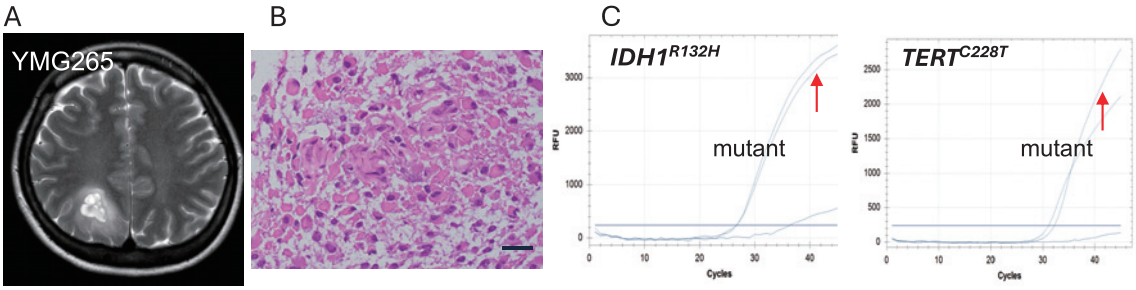
A 17-year-old male (YMG291) presented with memory disturbances. Diffuse abnormal signal intensity was detected on MRI. We performed an open biopsy for differential diagnosis. While the FS assessment did not determine whether this was a tumor, *i*-ID revealed *TERT*^{C228T} mutations and *IDH1/2* wild-type. Collectively, we diagnosed this tumor as a molecular feature of GBM, IDH wild-type, and further sampling was halted. Postoperative examination indicated diffuse glioma with *TERT*^{C228T} mutations, and the final integrated diagnosis was consistent with *i*-ID (Fig. 2G-I).

Discussion

In CNS malignant tumors, tumor classification and type have been revised every few years, together with advances in the field of neuro-oncology. Before the third edition of the WHO CNS tumors classification was published in 2007, the classification was mainly based on histological findings,[23] whereas the fourth edition, revised in 2016, introduced the presence of genetic abnormalities in the diagnosis.[24] In the fifth edition (WHO CNS5, revised in 2021), an integrated diagnosis combining histological and molecular diagnostics is required.[25] This means that diseases that were previously diagnosed and treated based on histology have shifted to a new era with molecular-based diagnosis, and treatment is provided according to molecular information. In the era of the CNS5, multi-omics analysis (reverse TR approach), such as intraoperative genomic assessment, has certainly contributed to a better understanding of tumor biology and promoted precision medicine.[7,26,27] However, these dry-lab approaches alone may have limitations in revealing how genomic and epigenomic alterations drive tumor initiation and progression if a reproducible model is not available.

PDX and PDC lines are useful for various wet-lab approaches such as genetic engineering, drug screening, and animal experiments. For example, using clinical and research materials, we have discovered several critical signaling pathways,[16,17] mutation-specific metabolic features,[18,28] and critical genomic alterations[19,29,30] that drive tumor progression and phenotypes in various CNS tumors. In addition, we have been referring drug sensitivity assays and genomic profiling for consideration in determining the treatment strategy (Fig. 2). These integrated systems with dry- and wet-lab approaches may support personalized therapy for individual patients.

During the past few decades, one of the major developments in the field of neuro-oncology has been the discovery of *IDH1* and *IDH2* mutation.[2,11] After this discovery, TR using highly reproducible models provided many insights into how genetic and epigenetic factors drive tumor formation and progression,[20,31-33] the therapeutic vulnerability of these gene alterations,[34-37] and selective therapeutic strategy for *IDH* mutant gliomas.[38-41] Targeting mutant *IDH1* or *IDH2* with a brain penetrant inhibitor improved progression-free survival in *IDH1/2* mutant low-grade (CNS WHO grade 2) glioma patients;[12] however, it did not show



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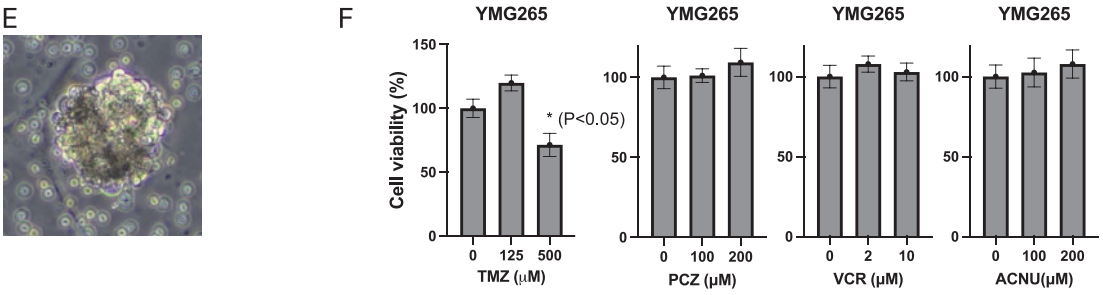
Cerebrum:	
Integrated diagnosis	Oligodendroglioma, IDH-mutant, and 1p/19q co-deleted, CNS WHO grade 3
Histopathological classification	Oligodendroglioma
CNS WHO grade	WHO grade 3
Molecular information	IDH1^{R132H}, Chromosome 1p/19q co-deleted, TERT^{C228T}, CDKN2A loss (-), MGMT methylated.



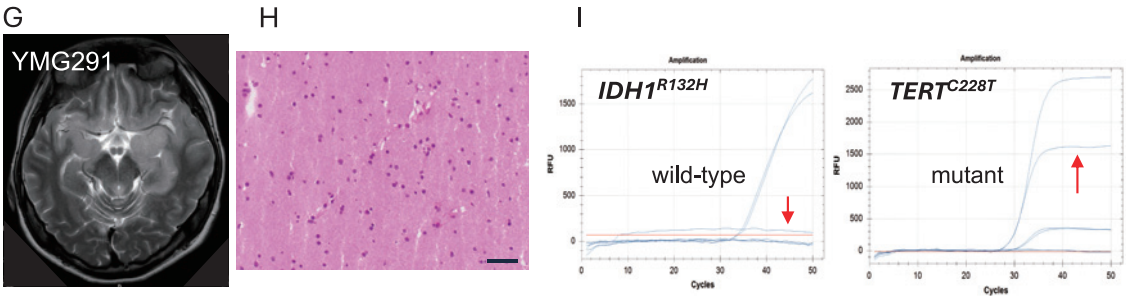


Fig. 2 Illustrative cases using translational research platform. A, T2-weighted magnetic resonance imaging indicating abnormal signal intensity on right occipital lobe. B, Frozen section imaging (hematoxylin and eosin staining). C, Intraoperative genotyping for *IDH1*^{R132H} (left, arrow) and *TERT*^{C228T} (right, arrow). D, Permanent integrated diagnosis in the present case. E, Primary cultured tumorsphere. F, Relative cell viability for indicated chemotherapeutic agents. TMZ, temozolomide; PCZ, procarbazine; VCR, vincristine; ACNU, nimustine. G, T2-weighted magnetic resonance imaging indicating diffuse abnormal signal intensity on the brain. H, Frozen section imaging (hematoxylin and eosin staining). I, Intraoperative genotyping for detecting *IDH1*^{R132H} (left, arrow) and *TERT*^{C228T} (right, arrow). Bars, 50 μm.

a durable response in recurrent or progressive glioma patients.[42] These findings were predicted by our prior TR using *IDH1* mutant glioma cell lines,[37] and in such unresponsive cases, synthetic lethal approaches, proposed by TR, are expected to be alternative therapeutic strategy.[34,36,39] However, we need to know the major gaps between basic research and clinical applications. For instance, PDX is not promising for assessing the immune system because immunosuppressed mice are used. Taken together, a combined approach using dry- and wet-lab techniques could develop a neuro-oncology field that may result in improved clinical outcomes.

Conclusions

In this study, I present our TR system for CNS malignant tumors. These models using multi-omics approaches may contribute to a better understanding of tumor biology and the development of novel therapeutic approaches for the poor prognosis of CNS tumors.

Acknowledgments

We thank our lab (Neurosurgical-Oncology lab) and neurosurgical members at Yokohama City University for supporting all clinical and TR efforts.

Funding Statement

This work was supported by Grant-Aid for Scientific Research C (22K09210 to KT).

Author Contributions

K.T.: study design, acquisition of material, writing the draft manuscript, and approval of the final manuscript.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Yokohama City University (YCU, Yokohama, Japan; A171130006 and B210300065). Written informed consent was obtained from all the patients and their families.

Conflicts of Interest Disclosure

The author declares no conflicts of interests.

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Corresponding author: Kensuke Tateishi, M.D., Ph.D.
Department of Neurosurgery, Yokohama City University, 3-9 Fukuura, Kanazawa, Yokohama, Kanagawa 236-0004, Japan.
e-mail: ktate12@yokohama-cu.ac.jp