Targeting Trends 2021 Review

TARGETED ABLATION WITH IB4-SAP

Selective ablation of IB4+ primary afferent neurons reduces mechanical and cold hyperalgesia in an EAE mouse model of multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Over 50% of patients with MS suffer from neuropathic pain (MSNP).[1,2] Patients also show poor response to analgesic drugs, prompting calls for a better understanding of underlying mechanisms.[3] A major tool to interrogate such mechanisms are rodent models of experimental autoimmune encephalomyelitis (EAE), which mimic the neuroimmune and somatosensory pathology of MS and MSNP.[3,4] While most studies have used EAE models to focus on CNS mechanisms, the contribution of primary afferent neurons (PANs) to MSNP is just now emerging.[5-7] For example, EAE sensitizes both small and medium-to-large diameter PANs leading to their hyperexcitability.[5] EAE also increases after hyperpolarization in small-diameter PANs leading to an increase in their firing of action potentials.[7] We chose to focus on the Isolectin B4 (IB4) subpopulation of small-diameter nonpeptidergic C-nociceptors because they mediate acute mechanical nociception and become hypersensitive after nerve injury.[8]

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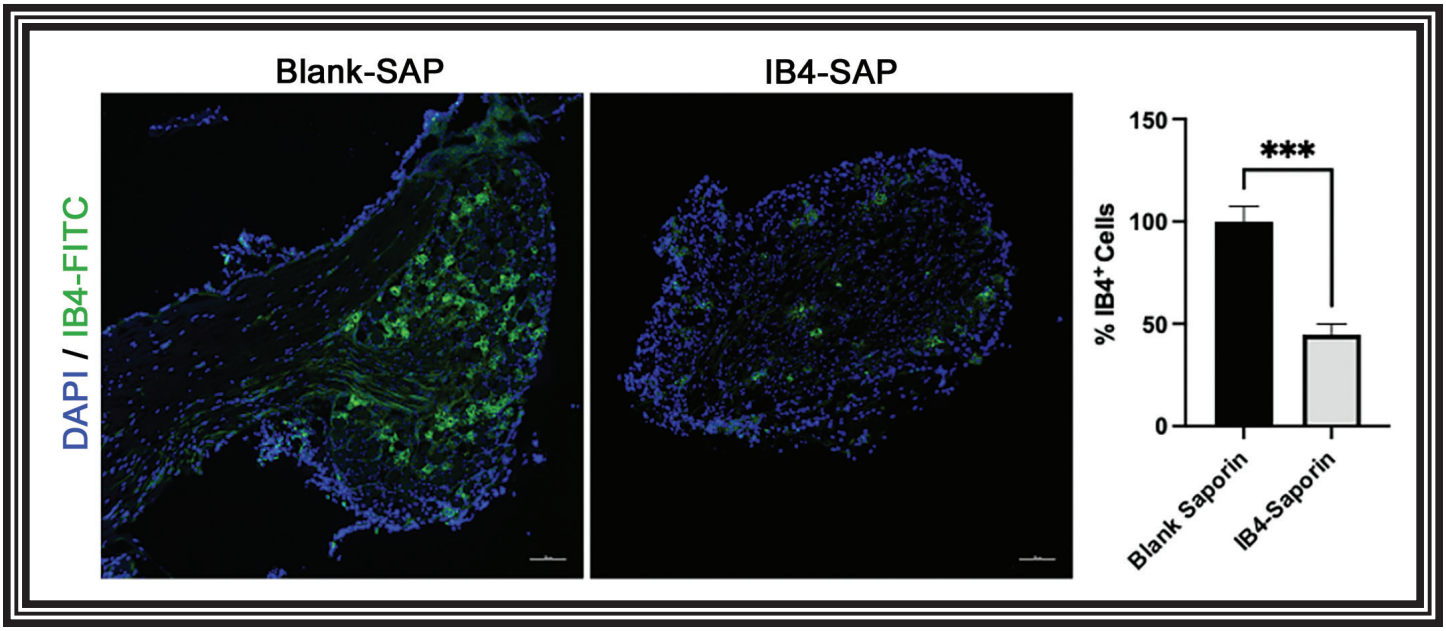

Figure 1: Intrathecal IB4-SAP (1.5 µg) significantly depleted nonpeptidergic C-fibers in lumbar DRG of B6 mice (representative IB4-FITC staining, scale bar = 100 µm; Bar graph represents the mean ± S.E.M. n=4; T-Test; ***p<0.001).

Targeted Ablation with IB4-SAP

(continued from page 1)

To test the hypothesis that IB4+ neurons in the dorsal route ganglion (DRG) contribute to hyperalgesia in EAE, we selectively ablated them with intrathecal injection (1.5 µg in a total volume of 5 µL) of saporin-conjugated IB4+ (**IB4-SAP, Cat. #IT-10**). Controls received 0.65 µg of blank saporin (**Blank-SAP, Cat #IT-21**). Figure 1 illustrates that IB4-SAP reduced IB4 staining by over 50% as compared to controls.

Next, we induced EAE in 10-week-old female mice with two subcutaneous injections of myelin oligodendrocyte glycoprotein (MOG35-55) peptide (100 µg) and/or complete Freund's adjuvant (CFA) containing 200 µg of heat-inactivated Mycobacterium tuberculosis H37Ra9 (4 mg/ml). As illustrated in Figure 2, EAE mice (but not naïve mice) given CFA or MOG+CFA developed mechanical (von Frey) and cold (acetone) allodynia, but this only persisted in MOG+CFA mice. IB4-SAP was administered 12 days later, and this reduced mechanical and cold hyperalgesia in MOG+CFA mice but had no effect in naïve or CFA-treated controls (Fig. 2).

These data suggest that nonpeptidergic IB4+ C-nociceptors contribute to the maintenance of MSNP. In future studies, we will explore the contribution of nonpeptidergic C-fibers to the induction of MSNP, as well as the contribution of large-diameter A-fibers.

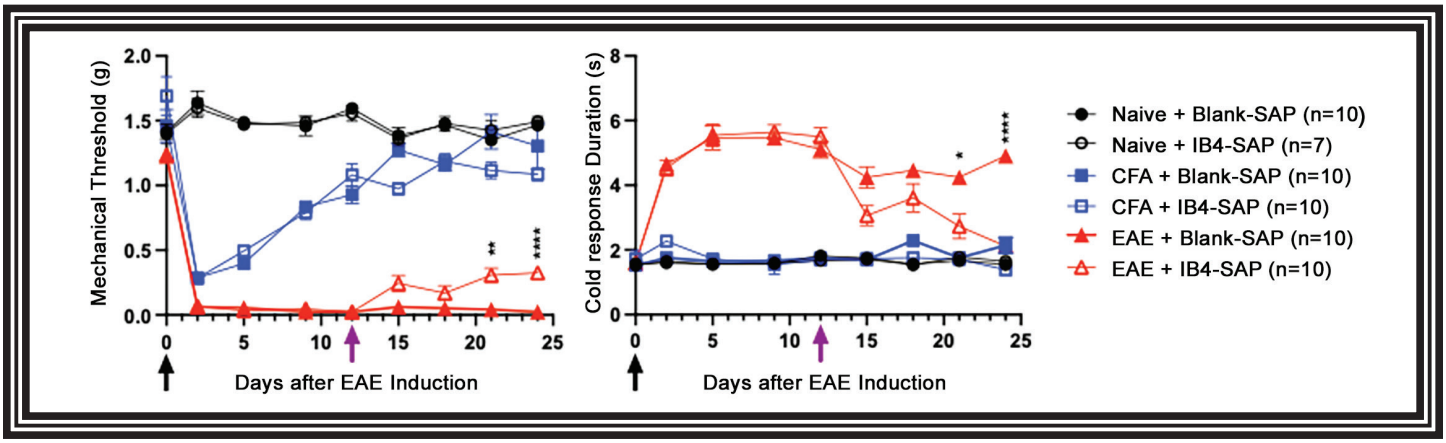

Figure 2: IB4-SAP significantly reduces mechanical (left) and cold (right) hypersensitivity in EAE. Black arrow = 1st EAE immunization, Purple arrow = Blank-SAP/IB4-SAP (Two-way ANOVA; *EAE+saporin vs. EAE+IB4-SAP; Bar represents mean ± S.E.M.)

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2021 Scientific References

Targeted Toxins

Targeting agent attached to Saporin

IT-01 192-IgG-SAP

Disruption of basal forebrain cholinergic neurons after traumatic brain injury does not compromise environmental enrichment-mediated cognitive benefits. Moschonas EH et al. Brain Res 1751:147175, 2021.

Objective: To determine if basal forebrain cholinergic neurons are important mediators of environmental enrichment (EE)-induced benefits after traumatic brain injury.

Summary: These data show that despite significant medial septal ChAT+ cell loss, the EE-mediated benefit in cognitive recovery is not compromised.

Dose: 0.22 µg/1.0 µL 192-IgG-SAP was infused over 5 min at a rate of 0.2 µL/min.

Aberrant functional connectivity of basal forebrain subregions with cholinergic system in short-term and chronic insomnia disorder. Ma X et al. J Affect Disord 278:481-487, 2021.

Summary: Previous animal studies have identified the cholinergic basal forebrain (CBF) as a crucial structure in sleep-wake cycle modulation and lesion or inactivation of the BF has been found to increase delta electroencephalogram activity, disturb behavioral arousal, and reduce sleep. They reference Kaur et al. using 192-IgG-SAP to lesion the CBF to examine the role of these neurons in sleep behavior.

See: Kaur S et al. J Neurosci 28:491-504, 2008.

Characterizing the neural substrate of reward with the use of specific brain lesions. Cromwell HC Fakhoury M (Ed.): The Brain Reward System. Neuromethods. 165, 2021. Humana, New York, NY

Summary: This review is focused on experimental lesions and work using the rodent model examining the neural substrates of reward processing. Saporin is listed as a neurotoxin used to target selective neuronal populations with success.

See: Pappas BA et al. Neurosci Biobehav Rev 27(4):365-376, 2003.

Acetylcholine from the nucleus basalis magnocellularis facilitates the retrieval of well-established memory. Soma S et al. Neurobiol Learn Mem 107484, 2021.

Summary: The authors tested the effect of a cholinesterase inhibitor, donepezil, on the retrieval of memory after a long no-task period in extensively trained rats. The results suggest that acetylcholine released from the NBM contributes to the retrieval of well-established memory developed by a daily routine.

Dose: Cholinergic neurons of the nucleus basalis magnocellularis (NBM) were lesioned with 192-IgG-SAP. NBM-lesioned rats showed severely impaired task initiation and performance. These abilities recovered as the trials progressed, though they never reached the level observed in rats with intact NBM. Saline with or without 192-IgG-SAP (0.3 µg in 1 µL, per site) was bilaterally injected into 2 sites of the NBM.

Reduction of falls in a rat model of PD falls by the M1 PAM TAK-071. Kucinski A et al. Psychopharmacology (Berl) 238(7):1953-1964, 2021.

Summary: In addition to the disease-defining motor symptoms, patients with Parkinson's disease (PD) exhibit gait dysfunction, postural instability, and a propensity for falls. The muscarinic M1-positive allosteric modulator (PAM) TAK-071 improves the attentional performance of rats with BF cholinergic losses. The authors previously developed a rodent model of PD falls by demonstrating that rats with dual basal forebrain cholinergic and mediodorsal striatal dopamine losses ("DL rats") exhibit a heightened fall rate when required to traverse dynamic surfaces. This study tested the hypothesis that TAK-071 reduces fall rates in DL rats.

Dose: Rats received bilateral infusions of 192-IgG-SAP (200 ng/µL; 0.8 µL/hemisphere) or an equal volume of artificial cerebral spinal fluid into the nucleus basalis and substantia innominata of the basal forebrain.

Fluoxetine and ketamine reverse the depressive but not anxiety behavior induced by lesion of cholinergic neurons in the horizontal limb of the diagonal band of broca in male rat. Chen L et al. Front Behav Neurosci 15:602708, 2021.

Summary: A lesion of horizontal limb of the diagonal band of Broca (HDB) cholinergic neurons and followed hippocampus damage may be involved in the pathogenesis of depression.

Dose: Injections of 192-IgG-SAP were made bilaterally into the HDB in a volume of 0.5 µL per side with a concentration of 0.5 µg/µL.

Lesions of the nucleus basalis magnocellularis (Meynert) induce enhanced somatosensory responses and tactile hypersensitivity in rats. Dezawa S et al. Exp Neurol 335:113493, 2021.

Summary: The authors used 192-IgG-SAP to produce a selective cholinergic lesion in the nucleus basalis of Meynert (NBM) of rats and investigated whether the NBM lesion led to tactile hypersensitivity in the forepaw. Results suggest that neuronal loss in the NBM diminishes acetylcholine actions in the S1, thereby enhancing the cortical representation of sensory stimuli, which may in turn lead to behavioral hypersensitivity.

Dose: The lesion group received injection of 0.3 µL of 192-IgG-SAP into the left nucleus basalis of Meynert (NBM).

Nanobody: a small antibody with big implications for tumor therapeutic strategy. Sun S et al. Int J Nanomedicine 16:2337-2356, 2021.

Summary: This Journal Club commentary focuses on the publication by Kalinchuk et al. 2008.

Dose: The author refers to work with 192-IgG-SAP published by Blanco-Centurion et al. 2006. This group investigated whether basal forebrain cholinergic neurons are involved in adenosine regulation of sleep. 6 µg of 192-IgG-SAP was administered to the lateral ventricle of rats.

See: Blanco-Centurion C et al. J Neurosci 26(31):8092-8100, 2006. Kalinchuk AV et al. Neuroscience 157:238-253, 2008.

Therapeutic agent delivery across the blood-brain barrier using focused ultrasound. McMahon D et al. Annu Rev Biomed Eng 23:89-113, 2021.

Summary: Review of the use of focused ultrasound, in combination with circulating microbubbles, can be used to transiently and noninvasively increase cerebrovascular permeability with a high level of spatial precision. For minutes to hours following sonication, drugs can be administered systemically to extravasate in the targeted brain regions and exert a therapeutic effect, after which permeability returns to baseline levels.

Dose: Shin et al. 2019, reported improved spatial memory following FUS+MB exposure in a rat model of cholinergic neuron degeneration. 192-IgG-SAP was injected bilaterally into the lateral ventricle (4 µl at a concentration of 0.63 µg/µl at a rate of 1 µl/min).

Specific phospholipid modulation by muscarinic signaling in a rat lesion model of Alzheimer's disease. Llorente-Ovejero A et al. ACS Chem Neurosci 12(12):2167-2181, 2021.

Summary: Using a rat model of BFCN lesion, this study evaluated the lipid composition and muscarinic signaling in brain areas related to cognitive processes. Results suggest that the modulation of specific lipid metabolic routes could represent an alternative therapeutic strategy to potentiate cholinergic neurotransmission and preserve cell membrane integrity in AD.

Dose: 192-IgG-SAP was administered via intraparenchymal infusion to selectively eliminate BFCN in the NBM.

Olfaction, cholinergic basal forebrain degeneration, and cognition in early Parkinson disease. Barrett MJ et al. Parkinsonism Relat Disord 90:27-32, 2021.

Summary: This study examined the relationship between olfaction, longitudinal change in cholinergic basal forebrain nuclei and their target regions, and cognition in early Parkinson's Disease.

See: Linster C et al. Behav Neurosci 115(4):826-833, 2001.

Electrical stimulation of the nucleus basalis of meynert: a systematic review of preclinical and clinical data. Nazmuddin M et al. Sci Rep 11(1):11751, 2021.

Summary: Deep brain stimulation (DBS) of the NBM (nucleus basalis of Meynert) in animal studies and the effects on behavioral and neurophysiological aspects are systematically reviewed. Translation of these outcomes to current clinical practice is hampered by the fact that mainly animals with an intact NBM were used, and most animals were stimulated unilaterally. Lee et al. (2016) addressed both of these issues using 192-IgG-SAP to lesion the NBM, which was stimulated thereafter.

Dose: Lee et al. lesioned the basal forebrain of rats through bilateral injections totaling 5 µg of 192-IgG-SAP into the lateral ventricle.

Cholinergic signaling, neural excitability, and epilepsy. Wang Y et al. Molecules 26(8):2258, 2021.

Summary: In this review, the authors briefly describe the distribution of cholinergic neurons, muscarinic, and nicotinic receptors in the central nervous system and their relationship with neural excitability and epilepsy. intraventricular administration of 192-IgG-SAP, which inhibits cholinergic projection to the hippocampus and cortex respectively, facilitates seizure induced by amygdala kindling

Dose: Ferencz et al. used 192-IgG-SAP (2.5 µg icv) to investigate the effect of eliminating cholinergic projections to the hippocampal formation and cerebral cortex on the induction of epilepsy through electrical stimulation of the rat brain. They determined that the loss of specific projections to the amygdala accelerates development of seizures.

See: Ferencz I et al. Eur J Neurosci 12:2107-2116, 2000.

Effects of age on sexually dimorphic food protection behavior associated with hippocampal cholinergic deafferentation. Osterlund Oltmanns JR et al. Neuroscience 2021 Abstracts P210/07, 2021. Society for Neuroscience, Virtual

Forebrain cholinergic plasticity in rats with chronic epilepsy induced by status epilepticus. da Costa C et al. U.PORTO Young Researchers Meeting, 2021

Summary: This poster had the following aims: 1) Evaluate the GABAergic population in the MS/DB in a chronic epilepsy model of kainic acid (KA)-treated rats. 2) Assess the GABAergic and cholinergic interconnectivity in the MS/DB in a chronic epilepsy model of kainic acid (KA)-treated rats. Results showed that outcomes were improved in rats receiving 192-IgG-SAP treatment as compared to Sham. Mortality: Sham - 50%; SAP - 0%.Recurrent motor seizures: Sham - 83%; SAP - 40%. Recurrent motor + EEG seizures: Sham - 100%; SAP - 50%.

Dose: 192-IgG-SAP was used to produce a moderate, but significant loss of septohippocampal cholinergic cells and to suppress their plasticity.

Evaluation of the Activity of Choline Acetyltransferase From Different Synaptosomal Fractions at the Distinct Stages of Spatial Learning in the Morris Water Maze. Storozheva ZI et al. Front Behav Neurosci 15:755373, 2021.

Objective: To examine the role of hippocampal and cortical ACh in the acquisition, consolidation, storage, retention and adaptive flexibility of new, recent and remote memory traces in spatial learning models.

Summary: The application of functional ablation or stimulation techniques is necessary. The approach used can be regarded as one of the possible ways of delineating temporal stages of spatial learning and could be applied in the studies of other signalling pathways.

Dose: Selective loss of septohippocampal and/or corticopetal cholinergic projections has been shown to cause attention deficit.

See: McGaughy J et al. Behav Neurosci 110:247-265, 1996. Lehmann O et al. Eur J Neurosci 18(3):651-666, 2003.

Placenta: A gold mine for translational research and regenerative medicine. Pethe P et al. Reprod Biol 21(2):100508, 2021.

Objective: To review recent studies regarding the therapeutic potential of human placenta-derived mesenchymal stromal/stem cells (hPMSCs) and their extracellular vesicles (EVs).

Summary: These studies demonstrate salutary effects of hPMSC-EVs on a range of different difficult-to-treat conditions like Duchenne Muscular Dystrophy, Parkinson's disease, acute kidney injury, etc., and therefore, it is imperative that these leads should be taken forward to clinical trials.

Dose: 8 µL of 192 IgG-saporin (0.63 µg/µL) were bilaterally injected into the ventricle to induce a dementia rat model.

The medial septum as a potential target for treating brain disorders associated with oscillopathies. Takeuchi Y et al. Front Neural Circuits 15:701080, 2021.

Summary: The medial septum (MS) may be a potential target for treating neurological and psychiatric disorders with abnormal oscillations (oscillopathies) to restore healthy patterns or erase undesired ones. The time-targeted strategy for the MS stimulation may provide an effective way of treating multiple disorders.

Dose: 192-IgG-SAP. The MS cholinergic neurons along with theta oscillations are known to be essential for memory because selective lesion of the cholinergic neurons resulted in spatial memory impairments (150 ng; Easton et al., 2011) (5.04 µg icv; Jeong et al., 2014). Orexin-SAP. The enhanced gamma oscillations and altered PPI and auditory gating created by psychoactive drugs in rats were mediated by GABAergic neurons in the MS because they were abolished by ablation of these neurons by Orexin-SAP (140 ng total bilateral; Ma et al., 2012). mu p75-SAP. Anxious environment-induced type 2 theta oscillation and associated anxiety were shown to be dependent on the MS cholinergic neurons because lesion of MS cholinergic neurons reduced them (0.65 or 1.3 µg, bilateral; Nag et al., 2009).

Heart failure impairs mood and memory in male rats and down-regulates the expression of numerous genes important for synaptic plasticity in related brain regions. Parent MB et al. Behav Brain Res 414:113452, 2021.

Objective: To assess the effects of heart failure (HF) on genetic markers of synaptic plasticity in brain areas critical for memory and mood, and to assess the effects of severely reduced ejection fraction (≤40 %) on cognition regulation.

Summary: Present findings provide support for the growing consensus that HF is not only a neurohumoral cardiovascular problem but is also a disorder of mood and memory.

See: Dobryakova YV et al. Front Mol Neurosci 10:429, 2018. Dobryakova YV et al. Front Neurosci 13:146, 2019.

Chemogenetic inhibition of prefrontal projection neurons constrains top-down control of attention in young but not aged rats. Duggan MR et al. Brain Struct Funct 226(7):2357-2373, 2021.

Objective: To test the hypothesis that reduced PFC output would exert differential effects on attentional capacities in young and aged rats, with the latter exhibiting a more robust decline in performance.

Summary: There is a reduced efficiency of PFC-mediated top–down control of attention and cholinergic system in aging, and that activity of PFC output neurons does not reflect compensation in aged rats, at least in the attention domain.

See: Dalley JW et al. Cereb Cortex 14(8):922-932, 2004. Newman LA et al. J Neurosci 28:2642-2650, 2008. Maddux JM et al. Behav Neurosci 121(1):63-79, 2007.

Neural circuitry underlying REM sleep: A review of the literature and current concepts. Wang YQ et al. Prog Neurobiol 204:102106, 2021.

Summary: To investigate the role of the LC in sleep the authors injected 0.3 µl of 192-Saporin (Cat. IT-01) or anti-DBH-SAP (Cat. #IT-03) at 1 µg/µl. They also used 0.3 µl of orexin-SAP (Cat. #IT-20) at either 90 ng/µl or 60 ng/µl in a separate group of animals. The results indicate that orexin innervation to the pons plays a role in arousal from sleep.

See: Blanco-Centurion C et al. Eur J Neurosci 19(10):2741-2752, 2004.

Overexpression of nerve growth factor in the hippocampus induces behavioral changes in rats with 192IgG-saporin-induced cholinergic deficit. Dobryakova YV et al. Neurochem J 15:273-281, 2021.

Summary: Degeneration of septal cholinergic neurons caused by the immunotoxin 192-IgG-SAP produces a model of the pathological state that occurs in Alzheimer's Disease. This study investigated whether overexpression of NGF in the hippocampus, where septal neurons send their projections, may reduce the consequences of this damage. Data suggest that NGF overexpression in the hippocampus of rats may partly compensate some 192 IgG-SAP-induced impairments related to cholinergic deficit.

Dose: 192-IgG-SAP or an equivalent volume of PBS (4 µg/site) was administered bilaterally into the ventricles.

Cholinergic regulation of adult hippocampal neurogenesis and hippocampus-dependent functions. Madrid LI et al. Int J Biochem Cell Biol 134:105969, 2021.

Summary: In this review, the authors appraise the evidence linking the contribution of cholinergic signalling to the regulation of adult hippocampal neurogenesis and hippocampus-dependent functions.

Dose: A hallmark feature of all basal forebrain cholinergic neurons is the expression of high levels of the p75 neurotrophin receptor which can be precisely targeted using 192-IgG-SAP. Administration of 192-IgG-SAP (icv, 2.5 µg, Mohapel et al., 2005) resulted in significant impairment in adult hippocampal neurogenesis in rats. In contrast, a study which lesioned MS cholinergic neurons in mice reported no effect on baseline proliferation in the hippocampus. Mice received 3.6 µg of mu p75-SAP into each lateral ventricle (Ho et al., 2009). Although the number of surviving neurons was similar in both lesioned and control animals, most of the progenitor cells in the lesioned animals could not survive without cholinergic input.

Neurotoxic effects, mechanisms, and outcome of 192 IgG-Saporin lesions. Petrosini L et al. RM Kostrzewa (Ed.): Handbook of Neurotoxicity , 2021. Springer, Cham

Summary: 192-IgG-saporin selectively destroys basal forebrain cholinergic neurons that provide cholinergic input to the hippocampus, entire cortical mantle, amygdala, and olfactory bulb. Immunotoxic lesions by 192-IgG-saporin represent a valid animal model of Alzheimer's disease, given the degeneration of basal cholinergic system present in this pathology. The selective lesioning of cholinergic innervation by means of 192-IgG-saporin (injected i.p. or i.c.v.) is able to interfere with experience-dependent plasticity.

IT-03 Anti-DBH-SAP

A5 noradrenergic neurons and breathing control in neonate rats. Taxini CL et al. Pflugers Arch 473(6):859-872, 2021.

Summary: The authors investigated the participation of A5 noradrenergic neurons in neonates (P7-8 and P14-15) in the control of ventilation during hypoxia and hypercapnia. data suggest that noradrenergic neurons of the A5 region in neonate rats do not participate in the control of ventilation under baseline and hypercapnic conditions, but exert an inhibitory modulation on breathing variability under hypoxic challenge in early life (P7-8).

Dose: Anti-DBH-SAP (420 ng/µL) or saporin (SAP, control) was injected into the A5 region of neonatal male Wistar rats.

Pain and depression comorbidity causes asymmetric plasticity in the locus coeruleus neurons. Llorca-Torralba M et al. Brain 145(1):154-167, 2021.

Summary: There is strong comorbidity between chronic pain and depression. This study explores how this comorbidity occurs. The authors refer to published research that shows icv administration of anti-DBH-SAP or intra-LC administration of lidocaine dampened the evoked pain in conditions of long-term nerve-injury. However, icv injection of anti-DBH-SAP disrupts all noradrenergic nuclei (A1-A7), some of which contribute to sensorial hypersensitivity.

See: Brightwell JJ et al. Neuroscience 160:174-185, 2009. Marques-Lopes J et al. Brain Res 1325:41-52, 2010.

Role of A1/A2 neurons in the dysregulation of vasopressin release and dilutional hyponatremia in liver disease. Aikins A et al. FASEB J 35(1), 2021. Experimental Biology 2021 Meeting Abstracts

Summary: Experiments suggest that A1/A2 neurons could be involved in the increased plasma AVP seen in male BDL rats as well as the decreased plasma osmolality.

Dose: Selective lesioning of the supraoptic nucleus (SON)-projecting A1/A2 norepinephrine neurons was achieved using anti-DBH-SAP.

Developments in understanding diffuse noxious inhibitory controls: pharmacological evidence from pre-clinical research. Kucharczyk MW et al. J Pain Res 14:1083-1095, 2021.

Summary: This review discusses the pharmacological manipulation interrogation strategies that have been used to examine the functionality of diffuse noxious inhibitory controls (DNIC) and descending control of nociception (DCN).

Dose: Anti-DBH-SAP is one of the drugs tested to influence DNIC expression.They reference a publication that reported that icv injection of Anti-DBH-SAP abolished DCN expression. Anti-DBH-SAP (5 µg/5 µl) was injected in the left ventricle. Lesion of the LC resulted in failure of DNIC, an effect that mimics what is observed behaviorally after chronic TBI.

See: Irvine KA et al. Exp Neurol 333:113428, 2020.

Medullary noradrenergic neurons mediate hemodynamic responses to osmotic and volume challenges. Marques SM et al. Front Physiol 12:649535, 2021.

Summary: The study sought to determine the role of noradrenergic neurons in hypertonic saline infusion (HSI)-induced hemodynamic recovery. Findings show that together the A1 and A2 neurons are essential to HSI-induced cardiovascular recovery in hypovolemia.

Dose: Medullary catecholaminergic neurons were lesioned by nanoinjection of Anti-DBH-SAP (0.105 ng·nl−1) into A1, A2, or both (LES A1; LES A2; or LES A1+A2, respectively). Sham rats received nanoinjections of unconjugated saporin in the same regions.

Activation of spinal dorsal horn astrocytes by noxious stimuli involves descending noradrenergic signaling. Kawanabe R et al. Mol Brain 14(1):79, 2021.

Summary: Astrocytes are critical regulators of neuronal function in the central nervous system (CNS). Astrocytes in the spinal dorsal horn (SDH) increase intracellular Ca2+ levels following intraplantar injection of the noxious irritant, formalin, however the underlying mechanisms remain unknown. The authors investigated these mechanisms by focusing on the role of descending noradrenergic (NAergic). Activation of α1A-adrenaline receptors via descending LC-NAergic signals may be a common mechanism underlying astrocytic Ca2+ responses in the SDH evoked by noxious stimuli, including chemical irritants

Dose: Intrathecal treatment with Anti-DBH-SAP, which kills SDH-projecting NAergic neurons, attenuates formalin pain (5.0 µg/20 µl; Martin et al., 1999)

The role of BDNF-mediated neuroplasticity in cardiovascular regulation within the hypothalamus and brainstem. Thorsdottir DJ Univ Vermont, Dept Pharmacology 1387, 2021.

Summary: This PhD dissertation to determine the mechanism behind BDNF-mediated cardiovascular regulation.

Dose: Rats received bilateral NTS injections of vehicle or Anti-DBH-SAP, which selectively lesions catecholaminergic neurons. Treatment increased blood pressure in the GFP group but failed to affect blood pressure in the BDNF group.

IT-06 / IT-33 Mac-1-SAP

Role of microglia and astrocytes in spinal cord injury induced neuropathic pain. Miranpuri GS et al. Ann Neurosci 28(3-4): 219-228, 2021.

Summary: Given the severity and incapacitating effects of spinal cord injury neuropathic pain (SCINP), it is imperative to study the pathways involved and find new therapeutic targets in coordination with stem cell research, and to develop a new gold-standard in SCINP treatment. Chronic inflammation by microglia, when targeted with Mac-1-SAP, helps in pain reversal.

Can Src protein tyrosine kinase inhibitors be combined with opioid analgesics? Src and opioid-induced tolerance, hyperalgesia and addiction. Li Y et al. Biomed Pharmacother 139:111653, 2021.

Summary: In this review the authors discuss the important role Src protein tyrosine kinase plays in the adverse consequences of clinical application of opioids.

Dose: Intrathecal injection of Mac-1-SAP depletes microglial cells in the spinal dorsal horn and alleviates the loss of anti-nociception of morphine and prevents the decrease in morphine potency. This demonstrates that spinal microglial cells are necessary for morphine tolerance (15 µg; Leduc-Pessah et al., 2017).

Specific depletion of resident microglia in the early stage of stroke reduces cerebral ischemic damage. Li T et al. J Neuroinflammation 18(1):81, 2021.

The role of activated microglia during the development of ischemia remains controversial. The authors investigate the function of reactive microglia in the early stage of ischemic stroke. The results showed that specific depletion of microglia resulted in a significant decrease in ischemic infarct volume and improved performance in motor ability 3 days after stroke.

Dose: Mac-1-SAP is used to specifically eliminate microglia. Hippocampal slices from mouse were incubated with 13-nM Mac-1-SAP for 3 to 7 days.

See: Montero M et al. Brain Res 1291:140-152, 2009.

Reciprocal interactions between osteoclasts and nociceptive sensory neurons in bone cancer pain. Andriessen AS et al. Pain Rep 6(1):e867, 2021.

Summary: Current pharmacotherapies available for bone cancer pain are insufficient to provide safe and efficacious pain relief. The authors discuss the mechanisms used by cancer cells within the bone tumor microenvironment (TME) to drive bone cancer pain.

Dose: Microglial ablation using Mac-1-SAP (15 µg in 8.8 µl i.t.) and Saporin control (Cat. #PR-01, 8.8 µg in 8.8 µl), is sufficient to attenuate nerve injury-induced pain in male, but not female mice.

See: Sorge R et al. Nat Neurosci 18:1081-1083, 2015.

Neuroimmune interactions and osteoarthritis pain: focus on macrophages. Geraghty T et al. Pain Rep 6(1):e892, 2021.

Summary: The contribution of macrophages to osteoarthritis (OA) joint damage has garnered much attention in recent years. The authors discuss how macrophages participate in the initiation and maintenance of pain in OA and provide a review of preclinical models of OA.

Dose: Using the rat monoiodoacetate-induced (MIA) model of advanced knee OA, increased microglia were observed in the ipsilateral and contralateral dorsal horn by day 7; specific ablation of spinal microglia through intrathecal injections of Mac-1-SAP (15 mcg per intrathecal injection on days 0, 1, and 2), attenuated mechanical allodynia by days 5 and 7 after MIA.

See: Mousseau M et al. Sci Adv 4:1-12, 2018.

IT-07 SP-SAP

Chronic pain in dogs (Dolor crónico en el perro). Puente BR , 2021. Zaragoza Spain: Gruppo Asis Biomedia, S. L.

Summary: The author presents a thorough overview of aspects of canine chronic pain. He includes SP-SAP (Substance P-Saporin) as an experimental drug, “its use as an adjuvant analgesic in dogs with bone cancer has been studied,”

See: Brown DC et al. Anesthesiology 119(5):1178-1185, 2013.

IT-10 IB4-SAP

Development of a novel pipette tip-aided cell cloning method for the effective isolation of genome-edited porcine cell. Sato M et al. OBM Genetics 5(1):16, 2021.

Summary: Isolation of clonal cells from a single colony is an essential step in the process of obtaining pure populations of stably-transfected clones after gene transfer and the subsequent drug selection. In the present study, a novel, simple, and non-invasive technique for the isolation of cells from single colonies using a disposable pipette tip was developed.

A toxin-based, drug-free selection system involving IB4-SAP was employed in the present study.

Antiplexin D1 antibodies relate to small fiber neuropathy and induce neuropathic pain in animals. Fujii T et al. Neurol Neuroimmunol Neuroinflamm 8(5):e1028, 2021.

Summary: NeP patient-derived plexin D1-IgG selectively binds to isolectin B4-positive unmyelinated C-fiber type small DRG neurons that sense mechanical pain.

See: Tarpley JW et al. Brain Res 1029(1):65-76, 2004.

Studying human nociceptors: from fundamentals to clinic. Middleton SJ et al. Brain 144(5):1312-1335, 2021.

Summary: The authors injected 5 µg of IB4-SAP into the sciatic nerve in the left thigh. Lesioned animals displayed attenuated NGF-induced hyperalgesia, as well as differences in other pain-model markers.

See: Tarpley JW et al. Brain Res 1029(1):65-76, 2004.

Nociception impedes grasping recovery in the spinal cord injured rat. Walker J et al. Neuroscience 2021 Abstracts P372/04, 2021. Society for Neuroscience, Virtual

Contribution of small diameter non-peptidergic primary afferent neurons to central neuropathic pain in a new, more clinically relevant mouse model of multiple sclerosis. Nguyen KL et al. Neuroscience 2021 Abstracts P377/07, 2021. Society for Neuroscience, Virtual

IT-11 SSP-SAP

Possible contribution of cerebellar disinhibition in epilepsy. Ming X et al. Epilepsy Behav 118:107944, 2021.

Summary: The authors hypothesize that loss of inhibition from the cerebellum can lead to cortical activation and seizures. An animal study showed microinjection of SSP-SAP produced a selective ablation of hippocampal inhibitory interneurons in vivo and a highly focal disinhibition. These results also demonstrate that the “epileptic” pathophysiology produced by experimental status epilepticus or head trauma can be replicated by focal interneuron loss, without involving principal cell loss and other interpretive confounds inherent in the use of global neurologic injury models.

See: Martin JL et al. J Comp Neurol 436:127-152, 2001. Chun E et al. Epilepsia 60(5):e52-e57, 2019.

PARVing the way to cap translation for seizure control. Gross C Epilepsy Curr 21(5):360-362, 2021.

Summary: Loss of GABAergic interneurons leads to spontaneous recurrent seizures that persist over months if the amount and spatial spread of initial inhibitory neuron loss is sufficient.

Dose: Intrahippocampal injections of SSP-SAP (0.4 ng/10 nL) were performed using a 0.5-µL Neuros Syringe lowered into four hippocampal sites along both the transverse and longitudinal hippocampal axes bilaterally.

See: Chun E et al. Epilepsia 60(5):e52-e57, 2019.

IT-12 Dermorphin-SAP

The role of the patch compartment of striatum in reward-driven behaviors. Ahn J et al. Neuroscience 2021 Abstracts P747/06, 2021. Society for Neuroscience,

IT-14 CTB-SAP

CSF-CN contributes to cancer-induced bone pain via the MKP-1-mediated MAPK pathway. Chen P et al. Biochem Biophys Res Commun 547:36-43, 2021.

Summary: Cerebrospinal fluid-contacting nucleus (CSF–CN) has been reported to be involved in the development of neuropathic pain and inflammatory pain. This study aimed to see whether it also has a role in cancer-induced bone pain (CIBP). Targeted ablation of CSF-CN dramatically aggravated pain sensitivity.

Dose: Injection via icv of CTB-SAP was performed to “knockout” the CSF-CN.

Nonsteroidal anti-inflammatory drug (ketoprofen) delivery differentially impacts phrenic long-term facilitation in rats with motor neuron death induced by intrapleural CTB-SAP injections. Borkowski LF et al. Exp Neurol 113892, 2021.

Objective: To determine the effect of ketoprofen delivery on enhanced phrenic long-term facilitation (pLTF)

Summary: pLTF was surprisingly attenuated in 7d CTB-SAP rats and enhanced in 28d CTB-SAP rats (both $p < 0.05$) following ketoprofen delivery.

Dose: Rats received bilateral intrapleural injections of CTB-SAP; 25 µg dissolved in PBS.

A systematic review of oropharyngeal dysphagia models in rodents. Kim HN et al. Int J Environ Res Public Health 18(9):4987, 2021.

Objective: To organize the rodent models of oropharyngeal dysphagia reported to date.

Summary: Applying and analyzing the treatment in rodent models of dysphagia induced from various causes is an essential process to develop symptom-specific treatments. The results of this study provide fundamental and important data for selecting appropriate animal models to study dysphagia.

Dose: CTB-SAP treated rats exhibited targeted hypoglossal motor neuron death; decreased hypoglossal motor output; and swallowing and lick deficits.

Exercise is neuroprotective on the morphology of somatic motoneurons following the death of neighboring motoneurons via androgen action at the target muscle. Chew C et al. Dev Neurobiol 81(1):22-35, 2021.

Objective: To determine where the necessary site of androgen action is for exercise-driven neuroprotective effects on induced dendritic atrophy.

Summary: Exercise following neural injury exerts a protective effect on motoneuron dendrites, which acts via androgen receptor action at the target muscle.

Dose: Motoneurons innervating the left vastus medialis (VM) muscle were selectively killed by intramuscular injection of cholera toxin-conjugated saporin (CTB-SAP).

Tongue and hypoglossal morphology after intralingual cholera toxin B-saporin injection. Lind LA et al. Muscle Nerve 63(3):413-420, 2021.

Objective: To evaluate tongue morphology and ultrastructural changes in hypoglossal neurons and nerve fibers in an inducible rat model of dysphagia.

Summary: Preliminary results indicate this model may have translational application to a variety of neurodegenerative diseases resulting in tongue dysfunction and associated dysphagia.

Dose: Rats assigned to the CTB-SAP group (n = 10) received 25 µg CTB-SAP to produce hypoglossal motor neuron death.

Utilization of pectoralis minor accessory inspiratory muscles in a rodent model of respiratory motor neuron loss. Borkowski L et al. The FASEB Journal 35 (S1), 2021.

Objective: To develop a model of selective respiratory motor neuron death to study how breathing is impacted and advance targeted therapeutic interventions.

Summary: The data suggest that the pectoralis minor muscles have an independent motor pool that can become recruited to assist in maintaining eupnea (normal respiration).

Dose: Adult male rats received bilateral CTB-SAP or control (CTB unconjugated to SAP) intrapleurally.

Clobetasol promotes neuromuscular plasticity in mice after motoneuronal loss via sonic hedgehog signaling, immunomodulation and metabolic rebalancing. Vicario N et al. Cell Death Dis 12(7):625, 2021.

Summary: The focal removal of confined populations of spinal MNs by injection of CTB-SAP has proven to be useful in mimicking respiratory dysfunction, dysphagia, and focal MN loss.

See: Lind LA et al. Muscle Nerve 63(3):413-420, 2021. Gulino R et al. Int J Mol Sci 20(6):1500, 2019. Nichols N et al. Exp Neurol 267:18-29, 2015. Gulino R et al. Eur J Neurosci 31(8):1423-1434, 2010.

How are adenosine and adenosine A2A receptors involved in the pathophysiology of amyotrophic lateral sclerosis?. Mori A et al. Biomedicines 9(8):1027, 2021.

Objective: To examine potential biomarkers and the acute symptomatic pharmacology, including respiratory motor neuron control, of adenosine A2A receptor antagonism, and to explore the potential of the A2A receptor as a target for Amyotrophic Lateral Sclerosis (ALS) therapy.

Summary: CTB-SAP is listed in a table of experimental animal models of ALS. Intrapleural CTB-SAP injected rats (neurotoxic model of respiratory motor neuron death).

See: Seven YB et al. Exp Neurol 323:113067, 2020.

Divergent receptor utilization is necessary for phrenic long-term facilitation over the course of motor neuron loss following CTB-SAP intrapleural injections. Borkowski LF et al. J Neurophysiol 126(3):709-722, 2021.

Objective: The authors tested the hypothesis that phrenic long-term facilitation (pLTF) following treatment with CTB-SAP is: 1) adenosine 2A (A2A) receptor-dependent at 7d; and 2) serotonin (5-HT) receptor-dependent at 28d.

Summary: This study furthers understanding of the contribution of differential receptor activation to pLTF and its implications for breathing following respiratory motor neuron death.

Dose: Male rats received bilateral, intrapleural injections of CTB-SAP or Saporin Control (25 µg).

IT-16 mu p75-SAP

Minocycline in neurodegenerative and psychiatric diseases: An update. Romero-Miguel D et al. Eur J Neurol 28(3):1056-1081, 2021.

Summary: Review includes the mouse animal model of neurodegenerative disease using mu p75-SAP. Biological Effects: Attenuation of cholinergic neurons loss, glial activation and transcription of pro-inflammatory mediators.

Dose: 45 mg/Kg, i.p.

See: Hunter CL et al. Eur J Neurosci 19(12):3305-3316, 2004.

Cholinergic modulation of sensory processing in awake mouse cortex. Jimenez-Martin J et al. Sci Rep 11(1):17525, 2021.

Objective: To decipher the timing and significance of acetylcholine actions.

Summary: Study provides new insights into how the cortex processes sensory information and how loss of acetylcholine, for example in Alzheimer's Disease, disrupts sensory behaviours.

Dose: Focal cortical injection of mu p75-SAP or Rabbit IgG-SAP (1.7 mg/ml, 0.3 µl total volume, rate 0.075 µl/minute).

Loss of cholinergic innervation differentially affects eNOS-mediated blood flow, drainage of Aβ and cerebral amyloid angiopathy in the cortex and hippocampus of adult mice. Nizari S et al. Acta Neuropathol Commun 9(1):12, 2021.

Summary: In this report, icv administration of mu p75-SAP resulted in significant death of cholinergic neurons and fibres in the medial septum, cortex and hippocampus of C57BL/6 mice. This study supports the importance of the interrelationship between cholinergic innervation and vascular function in the etiology and/or progression of cerebral amyloid angiopathy (CAA) and suggests that combined endothelial nitric oxide synthase (eNOS)/cholinergic therapies may improve the efficiency of Aβ removal from the brain and reduce its deposition as CAA.

Dose: mu p75-SAP (0.596 µg/µl) was injected into the left and right lateral ventricles.

The effect of nerve growth factor on supporting spatial memory depends upon hippocampal cholinergic innervation. Eu WZ et al. Transl Psychiatry 11(1):162, 2021.

Objective: To determine whether the supportive effect of NGF on learning and memory is specifically dependent upon intact hippocampal cholinergic innervation.

Summary: The results demonstrate that the hippocampal cholinergic system is required for maintaining spatial memory function, without having an impact on anxiety.

Dose: Twelve-week-old male C57BL/6Narl mice were used for hippocampal cholinergic denervation. Mice received bilateral injections into the hippocampus; 0.2 µg of mu p75-SAP was administered per site.

Engrafted stem cell therapy for Alzheimer's disease: A promising treatment strategy with clinical outcome. Salwa et al. J Control Release 338:837-857, 2021.

Objective: This review provides a detailed update on stem cell therapy (SCT) for Alzheimer's Disease (AD)

Summary: What future holds for SCT in the treatment of AD is summarized

Dose: Liu et al. injected 1.5 µg of mu p75-SAP into the medial septum.

See: Liu Y et al. Nat Biotechnol 31(5):440-447, 2013.

Tart cherry (fruit of prunus cerasus) concentrated powder (tccp) ameliorates glucocorticoid-induced muscular atrophy in mice. Ku SK et al. Medicina (Kaunas) 57(5):485, 2021.

Summary: Tart cherries have shown memory impairment lowering properties.

See: Matchynski JJ et al. J Med Food 16(4):288-295, 2013.

Cholinergic regulation of adult hippocampal neurogenesis and hippocampus-dependent functions. Madrid LI et al. Int J Biochem Cell Biol 134:105969, 2021.

See IT-01 192-IgG-SAP

The medial septum as a potential target for treating brain disorders associated with oscillopathies. Takeuchi Y et al. Front Neural Circuits 15:701080, 2021.

See IT-01 192-IgG-SAP

IT-20 Orexin-B-SAP

Medial parabrachial nucleus is essential in controlling wakefulness in rats. Xu Q et al. Front Neurosci 15:645877, 2021.

Summary: Lesions of the LC with 192-IgG-SAP have no significant effect on wakefulness in rats (Blanco-Centurion et al.). Only the Orexin-SAP lesion involved in the MPB region resulted in the dramatic decrease of wakefulness in rats (Fuller et al.).

See: Blanco-Centurion C et al. J Neurosci 27:14041-14048, 2007. Fuller P et al. J Comp Neurol 519(5):933-956, 2011.

The effects of orexin-A and orexin receptors on anxiety- and depression-related behaviors in a male rat model of post-traumatic stress disorder. Han D et al. J Comp Neurol 530(3):592-606, 2022.

Objective: To determine the role of the orexin system in mediating anxiety- and depression-related behaviors in PTSD.

Summary: Intracerebroventricular administration of orexin-A alleviated behavioral changes in a PTSD rat model and partly restored the increased levels of OX1R in the medial prefrontal cortex (mPFC). These results suggest that the orexin system plays a role in the anxiety- and depression-related symptoms observed in PTSD.

See: Kaur S et al. PLoS One 4:e6346, 2009.

Expert Opinion: Managing sleep disturbances in people with epilepsy. Nobili L et al. Epilepsy Behav 124:108341, 2021.

Summary: The authors suggest that the dose and timing of antiepileptic medications and other co-medications should always be optimized in order to improve nocturnal sleep and avoid daytime sedation.

See: Hasegawa H et al. Sleep Med Rev 52:101317, 2020.

The medial septum as a potential target for treating brain disorders associated with oscillopathies. Takeuchi Y et al. Front Neural Circuits 15:701080, 2021.

See IT-01 192-IgG-SAP

Neural circuitry underlying REM sleep: A review of the literature and current concepts. Wang YQ et al. Prog Neurobiol 204:102106, 2021.

See IT-01 192-IgG-SAP

IT-28 NPY-SAP

Targeting spinal neuropeptide Y1 receptor-expressing interneurons to alleviate chronic pain and itch. Nelson TS et al. Prog Neurobiol 196:101894, 2021.

Summary: Intrathecal administration of NPY-SAP reduced several operant and cognitive measures of Complete Freund's adjuvant (CFA)-induced allodynia, including responsiveness to cold temperatures, feeding interference, and an escape task, but did not interfere with systemic morphine-induced analgesia.

See: Wiley RG et al. Neuroscience 161:139-147, 2009. Nelson TS et al. Sci Rep 9(1):7248, 2019.

IT-31 CCK-SAP

Intact vagal gut-brain signalling prevents hyperphagia and excessive weight gain in response to high-fat high-sugar diet. McDougle M et al. Acta Physiol (Oxf) 231(3):e13530, 2021.

Objective: To assess the function of the vagus nerve lack specificity.

Summary: Intact sensory vagal neurons prevent hyperphagia and exacerbation of weight gain in response to a HFHS diet by promoting lipid-mediated satiation.

Dose: Rat nodose ganglia were injected bilaterally with either CCK-SAP or unconjugated saporin as a control.

From obesity to hippocampal neurodegeneration: Pathogenesis and non-pharmacological interventions. Lee TH et al. Int J Mol Sci 22(1):201, 2021.

Summary: This review provides insights into how chronic metabolic disorders, like obesity, could impair brain health and cognitive functions in later life. The authors reference the use of CCK-SAP into the nodose ganglia to impair spatial memory and contextual episodic memory.

See: Suarez AN et al. Nat Commun 9(1):2181, 2018.

Dissection and surgical approaches to the mouse jugular-nodose ganglia. Han W et al. STAR Protocols 2(2):100474, 2021.

Dose: Injected 0.5 µl of CCK-SAP (250 ng/µl) into the R-NG of VGlut2-ires-Cre mice.

See: Han W et al. Cell 175:665-678, 2018.

Learning of food preferences: mechanisms and implications for obesity & metabolic diseases. Berthoud HR et al. Int J Obes (Lond) 45(10):2156-2168, 2021.

Objective: This review focuses on postoral nutrient sensing and signaling as an essential part of the reward system that shapes preferences for the associated flavors of foods.

Summary: There is a critical role for the vagal gut-to-brain axis in motivation and reward. An implication for obesity treatment is that bariatric surgery may alter vagal function.

Dose: Han et al. injected 0.5 µl of CCK-SAP (250 ng/µl) into the R-NG of VGlut2-ires-Cre mice.

See: Han W et al. Cell 175:665-678, 2018.

IT-38 FGF-SAP

Efficacy and selectivity of FGF2-saporin cytosolically delivered by PCI in cells overexpressing FGFR1. Vikan AK et al. Cells 10(6):1476, 2021.

Summary: The authors evaluated the efficacy and selectivity of PCI of FGF2-saporin (FGF-SAP) in cells overexpressing FGFR1. The authors conclude that to prevent off-target effects of FGF-based toxins, it will be necessary to circumvent binding to HSPGs, for example by mutating the binding site of FGF2 to HSPGs.

Roles of the FGF-FGFR signaling system in cancer development and inflammation. Wiedlocha A et al. Cells 10(9):2231, 2021.

Objective: To highlight the latest advances in understanding the role of the FGF-FGFR signaling system in the development of neoplastic diseases and in the induction and maintenance of inflammation and its sequelae.

IT-40 Bombesin-SAP

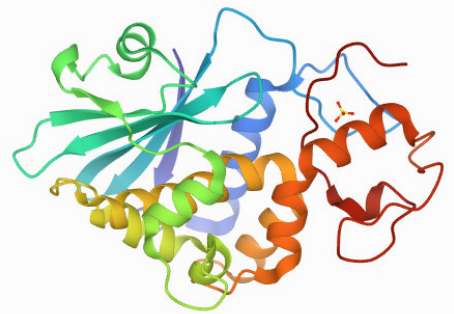
GRPR/extracellular signal-regulated kinase and NPRA/extracellular signal-regulated kinase signaling pathways play a critical role in spinal transmission of chronic itch. Liu X et al. J Invest Dermatol 141(4):863-873, 2021.

Summary: This study investigates whether there are certain key signaling molecules downstream of the recently identified peptides mediating itch in the spinal cord. Bombesin-SAP completely abolished extracellular signal-regulated kinase (ERK) activation. ERK was the most highly activated by their agonists BNP (Nppb, brain-derived natriuretic peptide) and octreotide. Nppb-SAP only partially reduced pERK in cervical spinal cord.

Central opioid receptors mediate morphine-induced itch and chronic itch via disinhibition. Wang Z et al. Brain 144(2):665-681, 2021.

Summary: Itch is a common side effect of opioids, particularly as a result of epidural or intrathecal administration. Notably, morphine-elicited itch was suppressed by intrathecal administration of NPY and abolished by spinal ablation of GRPR+ neurons with intrathecal injection of Bombesin-SAP.

Dose: For ablation of GRPR+ neurons, mice were given an intrathecal injection of 400 ng Bombesin-SAP or Blank-SAP (control) 10 days before behavioral testing.



Saporin . . . the Scalpel Used in Molecular Surgery!

The molecular basis for IL-31 production and IL-31-mediated itch transmission: from biology to drug development. Kunimura K et al. Int Immunol 33(12):731-736, 2021.

Objective: To investigate the molecular mechanisms of how IL-31 is produced in helper T cells upon stimulation and transmits the itch sensation to the brain.

Summary: This review highlights recent findings that show the functional significance of endothelial PAS domain 1 (EPAS1) and neurokinin B (NKB) in the IL-31-induced itch sensation.

Dose: Neurons expressing the Nppb receptor were specifically ablated by intrathecal injection of Nppb-SAP. Treatment with Bombesin-SAP reduced IL-31-induced scratching.

See: Sakata D et al. J Allergy Clin Immunol 144(4):1130-1133, 2019.

A neuropeptide code for itch. Chen ZF Nat Rev Neurosci 22(12):758-776, 2021.

IT-42 Anti-ChAT-SAP

Striatal cholinergic interneurons exert inhibition on competing default behaviours controlled by the nucleus accumbens and dorsolateral striatum. Ashkenazi SL et al. Eur J Neurosci 53(7):2078-2089, 2021.

Objective: To determine whether cholinergic interneurons contribute to the competition between both ventral and dorsolateral control systems.

Summary: Findings indicate a central role of cholinergic interneurons in regulating motivational impact on striatally controlled behaviors.

Dose: Anti-ChAT-SAP was diluted to 0.5 µg/µl in phosphate buffer saline and 0.5 µl were injected in each injection site.

An acetylcholine-dopamine interaction in the nucleus accumbens and its involvement in ethanol's dopamine-releasing effect. Loftén A et al. Addict Biol 26(3):e12959, 2021.

Summary: Basal extracellular levels of dopamine within the nucleus accumbens are not sustained by muscarinic acetylcholine, whereas accumbal Cholinergic interneurons-ACh are involved in mediating ethanol-induced dopamine release.

Dose: Anti-ChAT-SAP or Rabbit IgG-SAP were infused at a flow rate of 0.05 µl/min for 10 min giving a total volume of 0.5 µl.

IT-46 Oxytocin-SAP

Oxytocin influences male sexual activity via non-synaptic axonal release in the spinal cord. Oti T et al. Curr Biol 31(1):103-114.e5, 2021.

Summary: Oxytocin directly activates SEG (Spinal Ejaculation Generator)/GRP (Gastrin-Releasing Peptide) neurons via OXTRs (Oxytocin Receptors) and influences male sexual function in the rat lumbar spinal cord.

Dose: Oxytocin-SAP (4 or 40 ng) was infused slowly into the L3 and L4 spinal cord. Blank-SAP was used as control.

IT-47 Leptin-SAP

Centrally projecting edinger-westphal nucleus in the control of sympathetic outflow and energy homeostasis. Cano G et al. Brain Sci 11(8):1005, 2021.

Objective: To test the hypothesis that Edinger-Westphal nucleus (EWcp) integrates multimodal signals (stress, thermal, metabolic, endocrine, etc.) and modulates the sympathetic output simultaneously to multiple effector organs to maintain energy homeostasis under different conditions that require adjustments of energy demands.

Summary: Besides its role in feeding/metabolic control and addiction, EWcp is involved in several important functions that are ultimately related to different aspects of stress responses, as well as stress coping and adaptation.

See: Xu L et al. Gen Comp Endocrinol 177(3):296-304, 2012.

IT-69 Nppb-SAP

GRPR/extracellular signal-regulated kinase and NPRA/extracellular signal-regulated kinase signaling pathways play a critical role in spinal transmission of chronic itch. Liu X et al. J Invest Dermatol 141(4):863-873, 2021.

See IT-40 Bombesin-SAP

The molecular basis for IL-31 production and IL-31-mediated itch transmission: from biology to drug development. Kunimura K et al. Int Immunol 33(12):731-736, 2021.

See IT-40 Bombesin-SAP

IT-70 NMB-SAP

Sneezing pathway revealed. Whalley K Nat Rev Neurosci 22(8):455, 2021.

See: Li F et al. Cell 184(14):3762-3773.e10, 2021.

Sneezing reflex is mediated by a peptidergic pathway from nose to brainstem. Li F et al. Cell 184(14):3762-3773.e10, 2021.

Objective: To test the hypothesis that a neuronal population postsynaptic to nasal sensory neurons mediates sneezing.

Summary: Chemical activation of neuromedin B (NMB)-sensitive neurons elicits action potentials in caudal ventral respiratory group (cVRG) neurons and leads to sneezing behavior. This study delineates a peptidergic pathway mediating sneezing, providing molecular insights into the sneezing reflex arc.

Dose: Microinjection of NMB-saporin into the sneeze-evoking region abolished the sneezing responses to capsaicin and histamine.

IT-83 Anti-CD117-SAP

The biology of hematopoietic stem cells and its clinical implications. Skulimowska I et al. FEBS J 16192, 2021.

Objective: To review the opportunities and challenges of recent findings to improve the clinical use of hematopoietic stem cells (HSCs)

Summary: The authors describe new methods of HSC mobilization and conditioning for transplantation and highlight research that may lead to solutions for the limitations of HSC transplantation

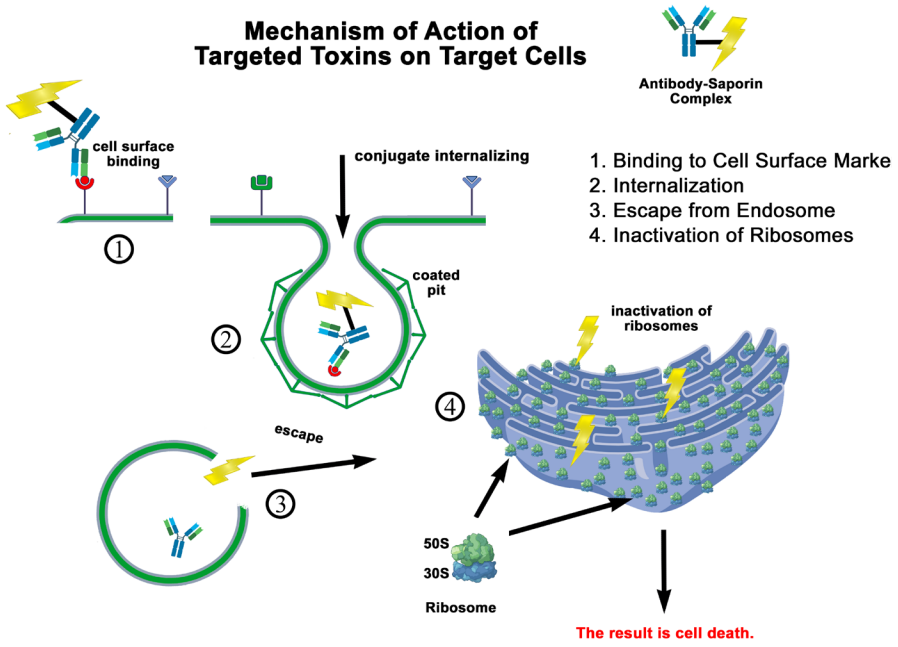
See: Palchaudhuri R et al. Nat Biotechnol 34:738-745, 2016.

IT-90 Ex4-SAP (GLP-1-SAP)

The deletion of glucagon-like peptide-1 receptors expressing neurons in the dorsomedial hypothalamic nucleus disrupts the diurnal feeding pattern and induces hyperphagia and obesity. Maejima Y et al. Nutr Metab (Lond) 18(1):58, 2021.

Summary: Feeding rhythm disruption contributes to the development of obesity. GLP-1 receptors (GLP-1R) are expressed in the dorsomedial hypothalamic nucleus (DMH) which are known to be associated with thermogenesis and circadian rhythm development. These findings suggest that GLP-1R expressing neurons in the DMH may mediate feeding termination.

Dose: Exenatide-SAP targets GLP-1R expressing cells. Injections of 0.1 µg/0.5 µl Ex4-SAP or 0.1 µg/0.5 µl Blank-SAP (control) were administered into the DMH.



Custom Conjugates

Identification of prostaglandin F2 receptor negative regulator (PTGFRN) as an internalizable target in cancer cells for antibody-drug conjugate development. Marquez J et al. PLoS One 16(1):e0246197, 2021.

See IT-67 Fab IgG-SAP

Integrin α10-antibodies reduce glioblastoma tumor growth and cell migration. Masoumi KC et al. Cancers (Basel) 13(5):1184, 2021.

Summary: The authors investigated the treatment effect of two antibodies that have been developed to target the protein integrin 10, which is present on the surface of Glioblastoma (GB) cells. Function-blocking integrin alpha10, beta1-antibodies inhibit GB tumor growth as well as the migration of GB cells. This further validates integrin alpha10, beta1 as a promising target in GB and suggests a novel therapeutic strategy for the treatment of GB and other high-grade gliomas.

Dose: Infusions of anti-integrin α10β1-SAP or Anti-ctrl-SAP were made icv (1 µg/2 L per infusion).

See: Thorén MM et al. Cancers (Basel) 11(4):587, 2019.

Control Conjugates

Control conjugates are non-targeted saporin conjugates

IT-21 Blank-SAP

Central opioid receptors mediate morphine-induced itch and chronic itch via disinhibition. Wang Z et al. Brain 144(2):665-681, 2021.

See IT-40 Bombesin-SAP

Oxytocin influences male sexual activity via non-synaptic axonal release in the spinal cord. Oti T et al. Curr Biol 31(1):103-114.e5, 2021.

See IT-46 Oxytocin-SAP

The deletion of glucagon-like peptide-1 receptors expressing neurons in the dorsomedial hypothalamic nucleus disrupts the diurnal feeding pattern and induces hyperphagia and obesity. Maejima Y et al. Nutr Metab (Lond) 18(1):58, 2021.

See IT-90 Ex4-SAP (GLP-1-SAP)

IT-35 Rabbit IgG-SAP

Cholinergic modulation of sensory processing in awake mouse cortex. Jimenez-Martin J et al. Sci Rep 11(1):17525, 2021.

See IT-16 mu p75-SAP

An acetylcholine-dopamine interaction in the nucleus accumbens and its involvement in ethanol's dopamine-releasing effect. Loftén A et al. Addict Biol 26(3):e12959, 2021.

See IT-42 Anti-ChAT-SAP

IT-67 Fab IgG-SAP

Identification of prostaglandin F2 receptor negative regulator (PTGFRN) as an internalizable target in cancer cells for antibody-drug conjugate development. Marquez J et al. PLoS One 16(1):e0246197, 2021.

See IT-48 Fab-ZAP

Saporin

Cyclodextrins based delivery systems for macro biomolecules. Liu J et al. Eur J Med Chem 212:113105, 2021.

Summary: This review recognizes Saporin as a targetable delivery system.

See: He X et al. Adv Funct Mater 29(50):1906187, 2019.

Short oligoalanine helical peptides for supramolecular nanopore assembly and protein cytosolic delivery. Pazo M et al. RSC Chem Biol 2:503-512, 2021.

Summary: Dose response transport experiments with MP1 (0–50 μM) in HeLa cells in the presence or absence of saporin (10 μg mL−1) showed a respectable 70% toxicity enhancement for MP1 and perfect cell viability for the peptide alone in the absence of the toxin.

Saporin, a polynucleotide-adenosine nucleosidase, may be an efficacious therapeutic agent for SARS-CoV-2 infection. Arslan I et al. SLAS Discov 26(3):330-335, 2021.

Summary: This mini-review focuses on how saporin-based targeted toxins may be efficacious therapeutic agents for SARS-CoV-2 infection.

Aptamer-driven toxin gene delivery in U87 model glioblastoma cells. di Leandro L et al. Front Pharmacol 12:588306, 2021.

Summary: A 26nt G-rich double-stranded DNA aptamer (AS1411) was integrated into a vector at the 5′ of a mammalian codon-optimized saporin gene, under CMV promoter. The gene encoding saporin is driven intracellularly by the glioma-specific aptamer that binds to cell surface-exposed nucleolin and efficiently kills target cells.

Phytochemistry and pharmacological activities of Saponaria officinalis L.: A review. Chandra S et al. Notulae Scientia Biologicae 13(1):10809, 2021.

Summary: Saponaria officinalis is an important medicinal and ornamental plant. Different saporins found in the species cause cytotoxicity of various cell lines and thereby play an important role in cancer treatment. Various kinds of saponins are synthesized by the species and exhibit anticancer, antimicrobial, antioxidant and antiinsecticide properties.

Magnetically navigated protein transduction in vivo using iron oxide-nanogel chaperone hybrid. Kawasaki R et al. Adv Healthc Mater 10(9):e2001988, 2021.

Summary: A magnetically guided *in vivo* protein transduction is demonstrated using magnetic nanogel chaperone (MC) composed of iron oxide nanoparticles and a polysaccharide nanogel, a protein carrier inspired by “catch and release” mechanisms of MCs. In an oral cancer model, MC-delivered magnetically targeted saporin decreased tumor volume without significant body weight changes and no regrowth of tumor at 3 months.

Different biological activities of histidine-rich peptides are favored by variations in their design. Lointier M et al. Toxins (Basel) 13(5):363, 2021.

Summary: The results show that saporin alone induces low levels of cell death, which is expected since it penetrates poorly into cells. In contrast, when mixed with LAH4 peptides, cell viability could be reduced by up to 80%.

Medullary noradrenergic neurons mediate hemodynamic responses to osmotic and volume challenges. Marques SM et al. Front Physiol 12:649535, 2021.

See IT-03 Anti-DBH-SAP

Editorial: Toxic plant proteins as experimental drugs for human pathologies. Polito L et al. Front Pharmacol 12:689924, 2021.

Summary: This Research Topic provides an update of the correlations between molecular damages induced by RIPs and the triggering of different cell death pathways.

Coiled coil-based therapeutics and drug delivery systems. Utterström J et al. Adv Drug Deliv Rev 170:26-43, 2021.

Summary: Coiled coils are abundant structural motifs found in many fibrous proteins and transcription factors and are often involved in assembly of higher order protein structures. The possibility to deliver drugs was evaluated by loading the highly potent protein toxin saporin (SAP) in the nanogels, resulting in a significant decrease in viability of MCF-7 breast cancer cells.

The effect of small molecule pharmacological agents on the triterpenoid saponin induced endolysosomal escape of saporin and a saporin-based immunotoxin in target human lymphoma cells. Wensley HJ et al. Biomedicines 9(3):300, 2021.

Summary: The authors used a number of pharmacological inhibitors of endocytic processes as probes to investigate the role played by saponin in the endolysosomal escape of fluorescently labeled saporin and a saporin based immunotoxin targeted against CD38 on human lymphoma and leukemia cell lines.

Plant-virus infection inhibitors: The great potential of Caryophyllales species. Duarte L et al. Physiol Mol Plant Pathol 113:101597, 2021.

Summary: This review proposes a model for the main mode of action hypotheses of viral infection inhibitors. The activity of two RIPs, in addition to PAP, against capped and uncapped viral RNAs has been reported. PAP, M. expansa RIP (ME1), and Saponaria officinalis RIP (saporin) depurinated capped TMV and brome mosaic virus (BMV, genus Bromovirus) RNAs, but did not depurinate uncapped luciferase RNA, indicating that in addition to PAP, other type I RIPs can distinguish between capped and uncapped RNAs.

Biomaterials for protein delivery for complex tissue healing responses. Dorogin J et al. Biomater Sci 9(7):2339-2361, 2021.

Summary: Delivery of saporin into MCF-7 breast cancer cells using pH-sensitive hyaluronic acid nanogels.

Reciprocal interactions between osteoclasts and nociceptive sensory neurons in bone cancer pain. Andriessen AS et al. Pain Rep 6(1):e867, 2021.

See IT-06 Mac-1-SAP

Divergent receptor utilization is necessary for phrenic long-term facilitation over the course of motor neuron loss following CTB-SAP intrapleural injections. Borkowski LF et al. J Neurophysiol 126(3):709-722, 2021.

See IT-14 CTB-SAP

Latest progress in the study of nanoparticle-based delivery of the CRISPR/Cas9 system. Yu M et al. Methods 194:48-55, 2021.

A brief history of saporin and its contributions to neuroscience. Shramm PA et al. Neuroscience 2021 Abstracts J002/11, 2021. Society for Neuroscience,

ZAP Conjugates

ZAP conjugates are non-targeted saporin conjugates that "piggyback" on to your primary targeting agent

IT-27 Streptavidin-ZAP

Activated factor X targeted stored in platelets as an effective gene therapy strategy for both hemophilia A and B. Wang D et al. Clin Transl Med 11(3):e375, 2021.

Summary: Treatment of hemophiliacs with inhibitors remains challenging, and new treatments are in urgent need.

Dose: A single dose of CD45.2-SAP (biotinylated Anti-CD45 mixed with Streptavidin-ZAP) enabled efficient engraftment of donor cells (> 90%) and full correction of sickle-cell anemia. (3 mg/kg iv; Palchaudhuri et al.).

See: Palchaudhuri R et al. Nat Biotechnol 34:738-745, 2016.

Efficacy and safety of anti-CD45-saporin as conditioning agent for RAG deficiency. Castiello MC et al. J Allergy Clin Immunol 147(1):309-320.e6, 2021.

Objective: To improve multi-lineage engraftment using non-genotoxic conditioning with Anti-CD45-Saporin.

Summary: Conditioning with Anti-CD45 antibody-drug conjugates may represent a novel and safe conditioning regimen for patients with RAG deficiency and other inborn errors of immunity.

Dose: Intravenous injection of Anti-CD45-SAP (3 mg/kg).

Autosomal recessive osteopetrosis: mechanisms and treatments. Penna S et al. Dis Model Mech 14(5):dmm048940, 2021.

Autosomal recessive osteopetrosis (ARO) is a severe inherited bone disease characterized by defective osteoclast resorption or differentiation. The authors review preclinical and proof-of-concept studies, such as gene therapy, systematic administration of deficient protein, in utero Hematopoietic stem cell transplantation (HSCT) and gene editing.

Dose: Efficacy in HSCT conditioning was demonstrated with CD45.2-SAP (biotinylated Anti-CD45 mixed with Streptavidin-ZAP). In mice, CD45.2–SAP preserved normal bone marrow architecture compared to total body irradiation, which instead reduced vascular integrity and bone marrow cellularity. Mice conditioned with CD45.2–SAP rapidly recovered their peripheral myeloid cells and had a survival advantage when exposed to infections (3 mg/kg iv; Palchaudhuri et al.). Additionally, conditioning with CD45.2–SAP resulted in significant chimerism after transplantation, even in a pathological mouse model (3 mg/kg iv; Castiello et al.).

See: Palchaudhuri R et al. Nat Biotechnol 34:738-745, 2016. Castiello MC et al. J Allergy Clin Immunol 147(1):309-320.e6, 2021.

Bone marrow-derived myeloid progenitors in the leptomeninges of adult mice. Koeniger T et al. Stem Cells 39(2):227-239, 2021.

Summary: Compared to bone marrow transfer after whole-body irradiation, chimerism developed more slowly in the CD45-SAP (biotinylated anti-CD45 mixed with Streptavidin-ZAP) model and only reached around 50% in the blood myeloid compartment 15 weeks after transplantation.

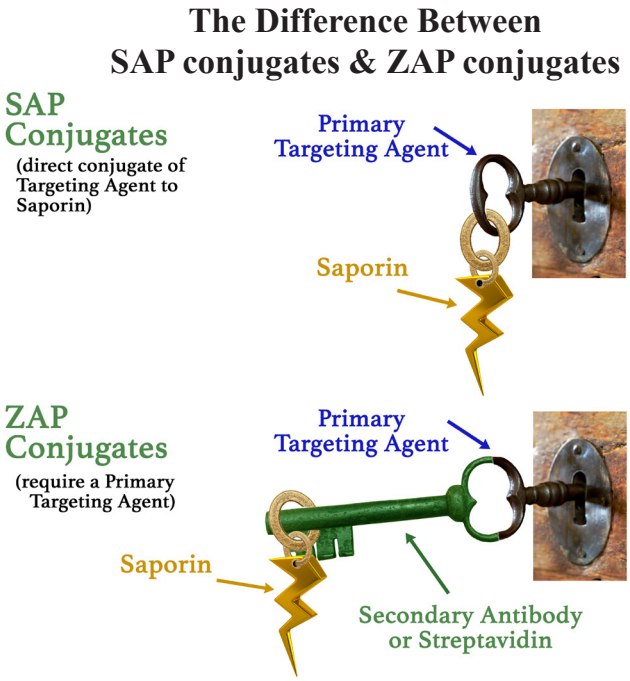
See: Palchaudhuri R et al. Nat Biotechnol 34:738-745, 2016.

IT-48 Fab-ZAP

Identification of prostaglandin F2 receptor negative regulator (PTGFRN) as an internalizable target in cancer cells for antibody-drug conjugate development. Marquez J et al. PLoS One 16(1):e0246197, 2021.

Summary: PTGFRN is a cell-surface protein that is upregulated in certain cancer types, including head and neck and, notably, pediatric medulloblastoma, an aggressive cancer with limited therapeutic options. With the selection of the mouse monoclonal antibody 33B7, the authors identified PTGFRN as a potential therapy target, and show that it is internalized by incubation with 33B7. Purified 33B7 antibody was sent to Advanced Targeting Systems where saporin was directly conjugated to the Fc region of 33B7 using their proprietary cleavable linker.

Dose: In a 96-well plate, 2000 cells/well were plated in triplicate in 100 µL of DMEM/F12 medium supplemented with 2.5% FBS, 0.4 ug/ml 33B7 antibody, and 0.9ug/ml of Fab-ZAP mouse. As an isotype control, cells were incubated with mouse Fab IgG-SAP as control (instead of 33B7) and Fab-ZAP.



Antibodies

AB-N27AP / AB-N28AP Angiotensin II receptor

Cardiac pathology in Mucopolysaccharidosis I mice: Losartan modifies ERK1/2 activation during cardiac remodeling. Gonzalez EA et al. J Inherit Metab Dis 44(3):740-750, 2021.

Objective: To investigate whether the pathways influenced by losartan and propranolol may modulate the cardiac remodeling process in MPS I mice.

Summary: Losartan and propranolol restore the heart function and could be used to ameliorate the cardiac disease in MPS I.

Usage: Western Blot 1:400

Baoyuan decoction (BYD) attenuates cardiac hypertrophy through ANKRD1-ERK/GATA4 pathway in heart failure after acute myocardial infarction. Meng H et al. Phytomedicine 89:153617, 2021.

Objective: To explore whether BYD plays a protective role against heart failure (HF) by attenuating cardiac hypertrophy (CH) via the ANKRD1-ERK/GATA4 pathway.

Summary: BYD ameliorates CH and improves HF through the ANKRD1-ERK/GATA4 pathway, providing a novel therapeutic option for the treatment of HF.

Usage: Western Blot

Comparative effects of angiotensin II on the contractility of muscularis mucosae and detrusor in the pig urinary bladder. Lim et al. Neurourol Urodyn 40:102-111, 2021.

Objective: To explore contractile actions of angiotensin II (AT2) on the muscularis mucosae (MM) of the bladder.

Summary: The MM appears to have great sensitivity to AT2, suggesting that MM may be the predominant target of contractile actions induced by AT2 in the bladder.

Usage: Induction of contractions with Anti-AT2R (1 nM – 1 μM). Immunofluorescence (10-100 pM).

Aqueous extract of Solanum nigrum attenuates Angiotensin-II induced cardiac hypertrophy and improves cardiac function by repressing protein kinase C-ζ to restore HSF2 deSUMOlyation and Mel-18-IGF-IIR signaling suppression. Lin HJ et al. J Ethnopharmacol 8:114728, 2021.

Objective: To investigate the ability of Solanum nigrum to attenuate Angiotensin II - induced cardiac hypertrophy and improve cardiac function through the suppression of protein kinase PKC-ζ and Mel-18-IGF-IIR signaling leading to the restoration of HSF2 desumolyation.

Summary: The data reveals the cardioprotective effect of S. nigrum inhibiting PKC-ζ with alleviated IGF IIR level in the heart that profoundly remits cardiac hypertrophy for hypertension-induced heart failure.

Usage: Western Blot

The protective effects of AT2R agonist, CGP42112A, against angiotensin ii-induced oxidative stress and inflammatory response in astrocytes: role of AT2R/PP2A/NFκB/ROS signaling. Bhat SA et al. Neurotox Res 39(6):1991-2006, 2021.

Objective: To evaluate the role and molecular mechanism of AT2R agonist CGP against Angiotensin II-induced astrocytic activation in primary astrocytes, and in a rat model of hypertension.

Summary: AT2R activation by CGP abrogated Ang II-induced astrocytic activation, by mitigating the ROS production, mitochondrial dysfunction, IκB-α degradation, NFκB nuclear translocation, and release of TNF-α in astrocytes.

Tetrahydrocurcumin ameliorates kidney injury and high systolic blood pressure in high-fat diet-induced type 2 diabetic mice. Sangartit W et al. Endocrinol Metab (Seoul) 36(4):810-822, 2021.

Objective: To investigate the protective effect of tetrahydrocurcumin (THU) on intrarenal RAS expression, kidney injury, and systolic blood pressure (SBP) in high-fat diet (HFD)-induced type 2 diabetic mice.

Summary: THU alleviated kidney injury in mice with HFD-induced type 2 diabetes, possibly by blunting the activation of the intrarenal RAS/nicotinamide adenine dinucleotide phosphate oxidase IV (NOX4)/monocyte chemoattractant protein 1 (MCP-1) axis and by lowering the high SBP.

Usage: Western Blot (1:1000)

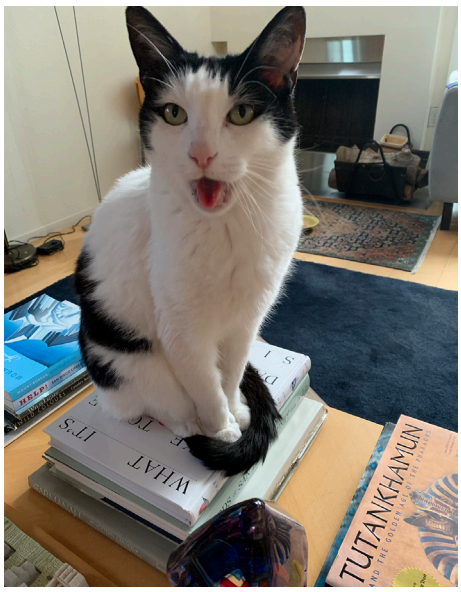
AB-T044A Anti-Conjugated Trans-4-Hydroxy-L-Proline

The HIF-1α antisense long non-coding RNA drives a positive feedback loop of HIF-1α mediated transactivation and glycolysis. Zheng F et al. Nat Commun 12(1):1341, 2021.

Objective: To investigate the contributions of HIF-1 in regulating glycolysis of cancer cells under hypoxia

Summary: HIF Antisense LncRNA (HIFAL) has a critical regulatory role in HIF-1-driven transactivation and glycolysis, identifying HIFAL as a therapeutic target of cancer treatment.

Usage: Western blot (1:500)



Kermit, just say “Ahhh.”

Top 20 Targeting Tools of 2021

Top 5 Targeted Toxins

1. CTB-SAP (Cat. #IT-14)
   targets cells expressing GM1 receptor
2. Mac-1-SAP mouse/human (Cat. #IT-06
   targets cells expressing Mac-1 (CD11b) receptor
3. Anti-CD103-SAP (Cat. #IT-50)
   targets cells expressing CD103
4. Anti-DBH-SAP (Cat. #IT-03)
   targets cells expressing rat dopamine beta-hydroxylase
5. mu p75-SAP (Cat. #IT-16)
   targets cells expressing mouse p75NTR

Top 5 ZAP Products

1. Streptavidin-ZAP (Cat. #IT-27)
   Uses your biotinylated material
2. Fab-ZAP human (Cat. #IT-51)
   Uses your primary human monoclonal antibody
3. Fab-ZAP mouse (Cat. #IT-48)
   Uses your primary mouse monoclonal antibody
4. FabFc-ZAP human (Cat. #IT-65)
   Uses your primary human monoclonal IgG antibody
5. Fab-ZAP rat (Cat. #IT-55)
   Uses your primary rat monoclonal IgG antibody

Top 5 in Number of Publications

1. 192-IgG-SAP (Cat. #IT-01)
   targets cells expressing rat p75NTR
2. CTB-SAP (Cat. #IT-14)
   targets cells expressing GM1 receptor
3. mu p75-SAP (Cat. #IT-16)
   targets cells expressing mouse p75NTR
4. Anti-DBH-SAP (Cat. #IT-03)
   targets cells expressing rat dopamine beta-hydroxylase
5. IB4-SAP (Cat. #IT-10)
   targets cells expressing α-D-galactopyranoside residues

Top 5 Antibodies

1. Melanopsin Rabbit Polyclonal, affinity-purified (Cat. #AB-N39)
2. NGFr (mu p75) Rabbit Polyclonal, affinity-purified (Cat. #AB-N01AP)
3. NGFr (ME20.4, p75) Mouse Monoclonal (Cat. #AB-N07)
4. NK-1 Receptor Rabbit Polyclonal, affinity-purified (Cat. #AB-N33AP)
5. trkA Rabbit Polyclonal (Cat. #AB-N03)