Journées du Campus d'Illkirch 2023

Talks

Developpment of an iPSC-derived bronchial epithelium model innervated by sensory neurons

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Résumé

Our project aims at obtaining an innervated bronchial epithelium model *in vitro*. It could serve as a model for the study of respiratory diseases including severe asthma, for which current treatments are still ineffective in a number of patients with severe clinical features.
We synthesised an extracellular matrix composed of a mixture of bovine collagen and chitosan. This matrix is first colonised by primary human bronchial fibroblasts and acts as a subepithelial mucosa. Sensory neurons derived from induced pluripotent stem cells (iPSCs) are cultured on the underside of the matrix and a bronchial epithelium derived from iPSCs from the same subjects is cultured on the surface, in an air/liquid interface, according to our previously described protocol (Ahmed et al., 2022).

We obtain an iPSC-derived bronchial epithelium presenting the main architecture and cell types of this tissue. It is based on an extracellular matrix thickened with 'endogenous' collagen secreted by the bronchial fibroblasts and forming a basal lamina. Saffron hematoxylin-eosin staining of paraffin sections shows a pseudostratified epithelium with different bronchial cell types, including ciliated cells and basal cells. Alcian blue and Schiff's periodic acid staining allows the visualisation of mucus cells. These results are confirmed by immunofluorescence. Ciliated cells are positive for the TUB IV, mucus cells for MUC5AC, basal cells are keratin 5+, club cells are CCSP+ , and we also observe neuroendocrine cells CHGA+. Partial innervation is demonstrated within the matrix by immunofluorescence of iPSC-derived sensory neurons (TUB**ß3**+). To improve this innervation, we added iPSCS-derived Schwann

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cells to this model to guide newly formed nerve fibres. Preliminary results indeed document an improvement of the innervation with the effective presence of longer nerve fibres within the matrix (TUBß3+). The expression of neuropeptides is confirmed by the presence of CGRP+ labelling.

In conclusion, we successfully obtained a 3D iPSC-derived human bronchial epithelium on a subepithelial mucosa. Reproducibility tests are currently underway to confirm the role of of Schwann cells on nerve spreading and their functionality.

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Nanoencapulation of NHC-platinum complexes: a promising strategy for glioblastoma treatment

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Résumé

Glioblastoma is a devastating brain tumor with poor prognosis despite the current standard of care (1). Platinum-based drugs, such as cisplatin and oxaliplatin, have limited efficacy due to secondary effects and resistance development over time against other types of cancer (2-3). Recently, platinum complexes bearing N-heterocyclic carbenes (NHCs) have emerged as promising candidates for glioblastoma treatment. They have been found to accumulate in the cytosol, nucleus and mitochondria contrary to oxaliplatin, which rather accumulates in the cytosol and nucleus (4). This is interesting because glioblastoma cancer stem cells rely on their high mitochondrial reserve to withstand treatment resulting in a relapse (5-6). NHC-platinum (NHC-Pt) complexes have been recently investigated *in vitro* and *in vivo* and they successfully eliminate glioblastoma stem cells (7).

In this study, we synthesized new NHC-Pt complexes with enhanced targeting of the mitochondria to simultaneously eradicate glioblastoma stem cells and differentiated cells. Also, to deal with poor solubility in water, a nanoencapsulation approach was undertaken. All NHC-Pt complexes demonstrate *in vitro* cytotoxicity against the glioblastoma stem cells (NCH421K) and the glioblastoma differentiated cells (U87-MG TMZ-sensitive). Our mechanistic investigations revealed that these novel compounds alter mitochondrial function and induce an atypical cell death. Different systems of nanoencapsulation have been formulated with the NHC-Pt which allowed us to get rid of DMSO for further investigations maintaining cytotoxicity.

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Mots-Clés: Glioblastoma, N Heterocylic Carbene, platinum, organometallic chemistry, cytotoxicity, cancer stem cells, nanoencapsulation.

Parsimonious Domain Adaptation for Cell Segmentation and its Theorical Interpretation

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Résumé

Electron microscopy imaging techniques allow biologists to obtain nanoscale slice volumes of cells in which biological structures can be statistically analyzed under the condition that the tedious task of segmentation can be automated. If a learned segmentation has good results on images obtained under the same acquisition process, variations in this acquisition can drastically affect the final image's features and can lead to a drop of segmentation performances. To tackle this problem, we propose an Unsupervised Domain Adaptation (UDA) framework based on Batch Normalization. Our method tested on mitochondria segmentation shows better results than state-of-the art methods and uses few computation resources, allowing a near real-time use. Furthermore, we propose a metric based on the Wasserstein distance to evaluate the effect of this normalization. We empirically show that this measure is log-linearly correlated with the drop of performances of the adapted segmentation and sheds light on the gain obtained by our UDA framework.

Mots-Clés: Domain Adaptation, Cell segmentation, Wasserstein distance, Optimal Transport

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Development of an antitumor vaccine approach based on the delivery of messenger RNA

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Résumé

Cancer immunotherapy is defined as the ability to mobilize the host's immune system to kill
cancer cells. It has recently taken a central role within mainstream oncology with the use of

immune checkpoint inhibitors and has shown unprecedented clinical responses in patients.

Despite this success, broad immunotherapy can result in severe adverse effects such as

autoimmunity, highlighting the need for new therapies. In the last decades, therapeutic

cancer vaccines have proven to be able to induce strong immune responses with little-to-no

adverse effects. Capable of eliciting exceptionally strong immune responses, RNA has

emerged as an attractive vaccine platform for cancer therapy. Thus, we propose to develop

innovative mRNA-based vaccine formulations that will allow the establishment of an effective

anti-tumor immune response.

Current mRNA vaccines are based on cationic lipid formulations which have been shown to

induce Reactive Oxygen Species and cell death on immune cells. They also contain small

amounts of poly(ethylene) glycol (PEG) that has been shown to be immunogenic, impairing

use of the same nanoparticles for future vaccines. Thus, developing suitable nextgeneration

mRNA-based nanoparticles for vaccination is a major challenge.

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As a part of our work, we designed cell penetrating peptides (CPPs) derived from viral fusogenic peptides. We investigated the ability of those CPPs to efficiently transfect mRNA into dendritic cells (DCs), the antigen presenting cells (APCs) which are the initiators of the immune response. By using mRNA coding for the reporter gene green fluorescent protein (GFP), we observe a strong transfection efficiency of various APCs (murine macrophages RAW264.7 and mouse DC2.4) and lower cytotoxicity as compared to the commercial transfection agent Lipofectamine (mixture of a cationic lipid and the fusogenic lipid DOPE). As RNA is also a danger signal to the immune system, we confirm the ability of our constructs to induce the activation of APCs by analyzing the overexpression of activation marker CD40 on the surface of those cells by flow cytometry.

We also confirmed the ability of our formulations to induce the presentation of the mRNA encoded antigen (i.e. ovalbumin) by the DCs and their ability to induce the activation of effector immune cells by co-culture with T-cells (responsible for the killing of cancer cells). Henceforward, we aim to ensure the ability of our formulations to induce immune reactions in vivo. Our molecules could thus represent an easy-to-formulate platform for mRNA vaccination that could be very interesting for cancer therapy.

Mots-Clés: Cancer, Vaccine, Immunology, Transfection, Cell penetrating peptide

MiR-30a-3p and miR-30e-3p influence tumour phenotype of head and neck squamous cell carcinoma by targeting TGF-beta/BMP signalling.

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Résumé

Head and Neck Squamous Cell Carcinoma (HNSCC) is the 6th most common cancer worldwide (Globocan, 2020). 70% of patients exhibit advanced HNSCC stage at diagnosis and their 5-year survival rate is less than 50% because therapeutic management is only based on the TNM classification and no reliable biomarkers for diagnosis or prognosis are available. In the last few years, miRNAs profile was studied in HNSCC and differences of expression between tumour vs healthy tissue were highlighted. Studies have suggested that miRNAs have the potential to become biomarkers (Sethi et al., 2014). Here, we studied members of the miR-30 family which act as antitumor miRNAs in HNSCC. We investigated the role of miR-30a-3p and miR-30e-3p expression on HNSCC relapses and clinical prognosis. Their expressions were analysed in 110 HNSCC HPV-negative, locally advanced tumours. miRNAs level of expression was correlated with clinical data and it showed that low level of miR-30a-3p and miR-30e-3p was correlated with higher risk of relapses and lower survival rate. Thus, these miRNAs have the potential to become biomarkers for relapses and prognosis. Next, we studied *in vitro* features of relapses and survival by overexpressing miR-30a-3p and miR-30e-p in HNSCC cell lines. Colony formation and apoptosis assays showed that survival was reduced when miRNAs were overexpressed in cells. In addition, spheroid evasion assay showed that migration was also reduced compared to control. Then, we wanted to determine the underlying pathway by which miRNAs affected these malignant biological functions. Screening TCGA database and evaluating connection of identified targets using STRING showed that miR-30a-3p and miR-30e-3p target several genes that happen to belong to the TGF-β network. Repression of this pathway was confirmed using RT-qPCR, western blot and immunofluorescence analyses. Pharmacological inhibition of the most affected targets, TGFBR1 and BMPR2, recapitulated effects observed by direct expression of miRNAs, suggesting that miRNAs exert antitumor activity through the inhibition of TGFBR1 and BMPR2. Beside their potential as biomarkers, miRNAs might be used to target one of the most important immunosuppressive pathway in HNSCC.

Mots-Clés: Head and neck squamous cell carcinoma, biomarker, miRNAs, TGFb signaling, tumor phenotype, relapses

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Development of analgesic tolerance to DOR agonist : involvement of GPRASP1 in the mechanism

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Résumé

The ∂-opioid receptor (DOR) is a G protein-coupled receptor (GPCR) involved in pain homeostasis. Activation of DOR by agonist gives an analgesic response in chronic pain models. However, repeated administrations of agonist lead to a rapid loss of their analgesic effect called analgesic tolerance. We are studying the molecular mechanisms involved in analgesic tolerance with two working hypothesis: tolerance at the cellular level could be caused by a rapid loss of activated DOR due to their degradation. Alternatively, in response to DOR stimulations, counteractive signaling pathways, pro-nociceptive, could be activated to restore pain homeostasis.
My project is to characterize the role of the intracellular GPCR-associated sorting protein 1 (GPRASP1) in the analgesic tolerance to DOR agonists. Indeed, GPRASP1 has been identified by our laboratory as a protein that interacts with DOR *in vitro* (1) and proposed by others to target DOR for degradation (2).

We have generated GPRASP1-deficient mice expressing a fluorescent DOR (DOR-eGFP) by crossing our GPRASP1-KO mice with knock-in DOR-EGFP mice (3). This unique mice model allows me to perform behavioural analysis of pain and to track DOR-eGFP with specific antibodies and by fluorescence.

I have shown that no analgesic tolerance to repeated DOR activation is developing in GPRASP1-KO mice but DOR are degraded to the same extent in WT and GPRASP1-KO mice. The elucidation of its involvement in tolerance may provide insight into the molecular

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adaptions to chronic activation of other GPCRs.

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Mots-Clés: delta, opioid receptor, analgesic tolerance, chronic pain, GPRASP1

MTM1 overexpression improves muscle function in a murine model of centronuclear myopathy associated with BIN1

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Résumé

Centronuclear myopathies (CNM) are severe muscle diseases associated with muscle weakness, as well as abnormal organelle positioning in myofibers. The main genes involved control membrane and lipid dynamics of muscle cells, particularly MTM1 and BIN1. Here, we evaluated the therapeutic potential of MTM1 protein overexpression in a *Bin1* KO murine CNM model. Ten weeks after neonatal and systemic injection of AAV-MTM1 viral vectors, treated mice showed normalized motor function and muscle strength, normal organelle positioning, and improved myofiber size. Intramuscular injection of AAV-MTM1 at a later stage of the disease only slightly improved the muscle strength defect but corrected histological defects. Overall, this study suggests that MTM1 overexpression represents an effective approach to antagonize muscle defects associated with CNM linked to BIN1.

Mots-Clés: Myopathy, AAV, therapy, mice, muscle

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Development and evaluation of innovative biomaterials with anti-inflammatory properties for the treatment of rheumatoid arthritis

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Résumé

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by progressive destruction of many joints. First-line treatments consist of two main strategies: targeting effector cells or limiting the chronic inflammation1. However, treatments are not without side effects. One of the alternatives considered to limit adverse reactions involves the use of biomaterials as vectors for therapeutic molecules2.

In this project, we aim to limit the underlying inflammation by specifically targeting one of the major cell types involved in the early stages of RA inflammatory development: macrophages.

We exploit the properties of albumin-based biomaterials recently developed within the laboratory (Patent application EP19306387, 2019)3. The materials are obtained by evaporation under non-denaturing pressure and temperature conditions. This process allows the formation of stable, versatile and biocompatible materials (membranes or microparticles) that can be loaded with a wide variety of numerous molecules, making them interesting vectors. We chose to incorporate two drugs already used in human inflammatory rheumatic diseases: the glucocorticoid dexamethasone and antibodies directed against pro-inflammatory TNF-α cytokine1.

We first studied the loading and release of dexamethasone inside the biomaterials respectively by ELISA assay and by flow-through dissolution method (USP4) followed by a quantification with HPLC. As the release profile is slow and progressive without burst effect, we then verified the anti-inflammatory potential of these materials in an *in vitro* model of LPS-stimulated murine macrophages. A significant reduction of NO and TNF-α was observed when dexamethasone was loaded inside the materials.

We therefore loaded our albumin-based biomaterials with human anti-TNF-α antibodies and studied TNF-α uptake as a second strategy. TNF-α capture is greatly enhanced when

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the materials are loaded with the antibodies in comparison to the unloaded ones.

Following these previous results, we combined the two treatments in the same biomaterial and checked their anti-inflammatory effect in a model of human PMA-stimulated macrophages. Improvement of TNF-α uptake is observed when the biomaterials are doubly charged.

In conclusion, formulations of albumin-based materials loaded with dexamethasone and antibodies have been developed. The resulting biomaterials have interesting anti-inflammatory properties and lead us to consider testing our material as an interesting way to deliver anti-inflammatory molecules in a RA mouse model4.

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Mots-Clés: biomaterials, albumin, inflammation, rheumatoid arthritis

PARP3 promotes myogenic differentiation and skeletal muscle function in cooperation with the histone methyltransferase EZH2.

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Résumé

PARP3, the third member of the PARP family, catalyzes a post-translational modification called mono(ADP-ribosyl)ation that is well defined by the host lab and others in the field for its role in DNA double-strand breaks repair, mitotic progression, tumor aggressiveness and astrocytic differentiation. My PhD project reveals an unexpected and unique role of PARP3 in myogenesis and skeletal muscle function. *In vitro*, we show that PARP3 increases during differentiation of C2C12 cells from myoblasts to myotubes. Crispr/Cas9-mediated disruption of *Parp3* in C2C12 cells significantly impairs their differentiation to myotubes and these abnormal cells display a disorganized cytoskeleton, degenerative mitochondria and accumulation of autophagic-like structures. These alterations are restored by the re-expression of a wild-type PARP3 in PARP3KO C2C12 cells, but not by the re-expression of a catalytically inactive mutant, therefore indicating the importance of the catalytic activity of PARP3 during myogenesis. Comparative transcriptomic analyses reveal altered expression of genes involved in muscle cell function, cell communication and cell identity. *In vivo*, histological analyses of the *Tibialis Anterior* (TA) skeletal muscles in mice show centrally nucleated fibers in PARP3KO mice which is associated with muscle weakness. Together these results describe a key role of PARP3 in myogenesis and muscle function in mice. Investigations of the mechanism involved reveal that PARP3 cooperates with the histone methyltransferase EZH2 to control the expression of specific genes essential for efficient differentiation.

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Deciphering the response of thienoguanosine (thG), a unique fluorescent nucleoside analogue towards UHRF1-SRA induced context-dependent and motif specific 5-methylcytosine base flipping interactions with oligonucleotides

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Résumé

DNA cytosine methylation by DNMTs acts as one of the leading characters in the context of epigenetic modifications. DNMT1 is recruited by UHRF1 where UHRF1 first recognizes 5mC (methyl cytosine) via its SRA domain which facilitates the approach of DNMT1 to cytosine in the complementary strand for further methylation. The binding pocket of SRA is selectively tailored in such a way that it can easily accommodate 5-mc after flipping and stabilized through non-specific interactions through amino acid residues. Conventional biophysical assay like X-ray crystallography or EMSA (Electrophoretic mobility shift assay) only provide the information regarding the static structure. In order to unravel the dynamics of base flipping process, real time monitorization is obligatory. In this context, fluorescent nucleoside analogues (FNAs) are well pronounced to probe structural and functional dynamics of nucleic acids during the interaction with other biological macromolecules. Thienoguanosine (thG), an isomorphic surrogate of natural guanosine is unique in its feature with standard quantum yield and long fluorescence lifetime compare to other common FNAs, makes it an excellent candidate to monitor base flipping mechanism induce by SRA. Base flipping of mC results loss of stacking with flanking nucleobases by which ground state population of thG shifts towards more emissive tautomer, causes 4 to 5 fold fluorescence increment. This insists us to further monitor the base flipping process in CpG context dependent and motif's specific sequences, labelled with thG. In all the cases thG's fluorescence along with absorption suggests base flipping is everywhere but variation on the fold increment of thG arises from the extent of stabilization of the flipping state. Stopped flow kinetic profile suggests that the whole base flipping process consists of three different steps: (a) initial binding; (b) flipping of mC to the binding pocket of SRA (c) stabilization of mC and rearrangement of amino acid residues to keep the DNA structure intact (B-strand). In an ideal condition oligo sequences having a CpG island and mC as flipping motif satisfy all the steps during flipping. But when mC is substituted by analogues derivative or CpG is replaced by CpA or CpT, rearrangement step either absent or much faster compare to sequences having CpG island.

Mots-Clés: Epigenetics, UHRF1, SRA, DNMT1, Fluorescent Nucleoside Analogues

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Modeling of the binding of neomycin B and its derivative into Aminoglycoside-Modifying Enzymes

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Résumé

Antibiotic resistance is a major challenge for humanity in terms of public health, economics, and ecology. The World Health Organization (WHO) has called for a global response with a "one health" approach including the development of new antibiotics. Our project falls within this framework and in collaboration with Pr. Weibel (LaSyROC, UMR 7177, CNRS-University of Strasbourg), Dr. Prévost (Virulence bactérienne précoce, UR 7290, Université de Strasbourg), and Dr. Ennifar (Architecture et réactivité de l'ARN, UPR 9002, CNRS-University of Strasbourg) for the optimization of neomycin, maximizing the bactericidal effect by minimizing antimicrobial resistance and toxicity.

Neomycin is an aminoglycoside with excellent antibacterial activities against gram-negative and gram-positive bacteria. It targets bacterial ribosomal RNA and thereby inhibits translation. Resistances to neomycin mostly involve Aminoglycoside-Modifying Enzymes deactivations (AME), which are the cause of approximately 60 to70% of acute resistances.1

The three-dimensional structures of five AMEs were modeled by homology for *E. coli* and *S. aureus* from clinical strains. For each model, neomycin and the synthetic derivative (HL_171) that showed the most interesting MIC activity were docked under constraints applied to ring II into the active site. All complexes were submitted to molecular dynamics simulation (3x 200ns). The binding modes, distances between the derivatives and the catalytic centers, and conformation of the active site were monitored and considered as indicators of the possible catalytic ability.

The objective of the simulations was to ascertain the impact of neomycin modifications on AMEs activity. Based on the analysis, two complexes exhibited a similar binding mode to neomycin B, inferring that the modifications do not affect metabolization. On the contrary, the remaining three complexes presented a dissimilar binding mode, suggesting that they could hinder AMEs metabolization activity.
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Mots-Clés: Aminoglycoside, AME, Antibiotic resistance, AMR, Modeling

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Quantitative affinity interactomics uncovers disease-related networks

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Résumé

The characterization of human protein-protein interactome networks is essential to understand biological processes in their full complexity in order to develop effective targeted therapies for disease treatment, but most of the high-throughput methods currently used in the field are qualitative. We have developed various quantitative affinity interactomics approaches that can not only identify the partners of proteins, but can also readily assess the equilibrium binding properties of these interactions with high accuracy and sensitivity. These techniques are suitable for measuring binding affinities between peptides and domains, or even full-length proteins taken directly from cell extracts. Through a case study of the BIN1 protein, I will show how a combination of these different strategies can be used to uncover the interactome underlying disease. BIN1 is a protein involved in membrane remodeling, mutated in myopathy, and is thought to recruit partner proteins to specific membrane tubules of muscle cells through its SH3 domain to promote excitation-contraction coupling. Loss of this domain has been shown to cause centronuclear myopathy, yet only a few partners of the SH3 domain of BIN1 have been identified. We used our different interactomics approaches to screen the proteome for new partners, quantify their interactions and identify their interacting peptide regions. Hundreds of novel interaction partners for the SH3 domain have been identified and quantified, and the binding mechanisms of most of them have been deciphered. Using the same strategy, we also explored the interactomic effects of missense variants localized in the SH3 domain of BIN1, which showed excellent coherence with cellular assays assessing the recruitment of these proteins to membrane tubules, demonstrating that quantitative interactomics can be a useful tool for validating loss-of-function mutations in proteins.

Related publications:
Zambo, B., Edelweiss, E., Morlet, B., Negroni, L., Ostergaard, S., Travé, G., Laporte, J., Gogl, G. (2023). Affinity-ranking BIN1 interactions underpinning centronuclear myopathy. *BioRχiv*. Weimer, K., Zambo, B., Gogl, G. (2023). Molecules interact. But how strong and how much? *BioEssays*, 2300007 Zambo, B., Morlet, B., Negroni, L., Travé, G.*, & Gogl, G.* (2022). Native holdup (nHU) to measure binding affinities from cell extracts. *Science Advances*, *8*(51), eade3828. Gogl, G., Zambo, B., Kostmann, C., Cousido-Siah, A., Morlet, B., Durbesson, F., Negroni, L., Eberling, P., Jané, P., Nominé, Y., Zeke, A., Østergaard, S., Monsellier, É., Vincentelli, R., & Travé, G. (2022). Quantitative fragmentomics allow affinity mapping of interactomes. *Nature Communications*, *13*(1), 5472.

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