"DYRK1A, related kinases & human disease"

Saint Malo, France

March 28 – April 1st, 2017

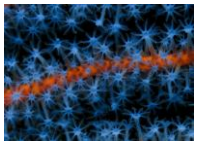
Phosphorylation by protein kinases is the most universally used mechanism by cells to control their structural proteins and enzymes. All major physiological phenomena are regulated by phosphorylation and many diseases are associated with abnormal phosphorylation. Therefore, the last four decades have seen considerable efforts in the study of functions and regulations of kinases in essentially all aspects of biology. In parallel, the search for pharmacological inhibitors of kinases has become a major area of research in the pharmaceutical industry for the discovery and development of new therapies.

DYRK1A ('dual specificity, tyrosine phosphorylation regulated kinase 1A') phosphorylates many substrates involved in signaling pathways. It plays key roles in mRNA splicing, gene transcription, cell survival, differentiation, endocytosis, neuronal development and functions. Abnormalities in DYRK1A dosage are associated with cognitive disorders observed in Down syndrome, Mental Retardation Disease 7 (MRD7) and Alzheimer's disease. DYRK1A plays key functions in pancreatic cells, cancer, inflammation, megakaryoblastic leukemia. Close orthologs are found in plants, yeast, algae and unicellular parasites.

The conference will focus on DYRK1A, DYRK1B, 2, 3, 4 and the closely related CLKs ('cdc2-like kinases). Presentations will cover all structural (crystal structures), regulatory and functional aspects of these kinases, their substrate specificity, functions at the cell and organism levels, the development of potent and selective pharmacological inhibitors and their potential therapeutic use as treatment of various human diseases.

Welcome to Saint Malo! We hope you will enjoy the conference program,
the interactions between participants, the local food, the scenery and
… the (unpredictable) weather!

Laurent **MEIJER**, Conrad **KUNICK** & Yann **HERAULT**
& Pauline **DE LAFFOREST**



PROGRAM

TUESDAY MARCH 28 – afternoon & evening

17.00 **Opening of Registration**
Le Palais du Grand Large
1 quai Duguay-Trouin
35 400 SAINT MALO

19.00 **Welcome address**
Le Palais du Grand Large
Salle Rotonde

19.10 **Welcoming drinks**

WEDNESDAY MARCH 29 – morning

8.00 – 9:00 **Registration**

SESSION 1. DYRK1A & Down syndrome — Chair: Jean-Maurice DELABAR

9.00 – 9.05 **WELCOME!**

9.05 – 9.30 **Yann HERAULT**
Challenging the role of DYRK1A in Down syndrome by studying the genotype and phenotype relationships in animal models

9.30 – 9.55 **Michel J. ROUX**
Retinal phenotypes in murine models of Down syndrome

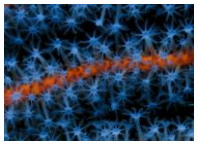
9.55 – 10.20 **Sungchan CHO**
A chemical with proven clinical safety rescues Down-syndrome-related phenotypes through DYRK1A inhibition

10.20 – 10.45 **Akiko KOBAYASHI**
Repression of DYRK1A restores impaired cortical development and abnormal learning behavior of Down syndrome mice

10.45 – 11.05 COFFEE AND TEA BREAK

11.05 – 11.30 **Thu Lan NGUYEN**
Deciphering the molecular mechanisms of action of pharmacological DYRK1A kinase inhibitors for therapeutic use in Down Syndrome preclinical models

11.30 – 11.55 **Cécile CIEUTA-WALTI**
Preclinical study of safety and biomarkers of efficacy of Epigallocatechin-3-gallate at three different doses in Wild-type and transgenic DYRK1A mice



SESSION 2. DYRK1A & CBS — Chair: Mariona L. ARBONES

11.55 – 12.20 **Jean-Louis GUEANT**
The influence of the metabolism of monocarbons on epigenomic mechanisms: the interacting role of the remethylation and transsulfuration pathways

12.20 – 12.45 **Gaëlle FRIOCOURT**
Identification of cystathione β-synthase pharmacological inhibitors to alleviate the intellectual deficiency of patients with Down syndrome

12.45 – 12.50 **Alice LEON**
Identification of a new function of Cystathionine β-Synthase (CBS) and characterization of its link with DYRK1A using *S. cerevisiae*

12.50 - 14.30 LUNCH

WEDNESDAY MARCH 29 – afternoon

SESSION 3. DYRK1A & Alzheimer's disease — Chair: Yann HERAULT

14.30 – 14.55 **Jérôme BRAUDEAU**
APPβ processing initiates full Tau pathology in a novel age dependent Alzheimer's disease rat model: GSK3β and DYRK1A levels during human-like AD progression

14.55 – 15.20 **Benoît SOUCHET**
Preventing DYRK1A catabolism in reactive astrocytes as a novel therapeutic approach to treat Alzheimer's Disease

15.20 – 15.45 **Jean-Maurice DELABAR**
DYRK1A, biomarker or target for Alzheimer's disease

SESSION 4. DYRK1A & Neurogenesis — Chair: Susana DE LA LUNA

15.45 – 16.10 **Mariona L. ARBONES**
Control of neuron numbers by DYRK1A: lessons from mouse models

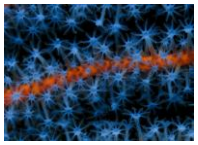
16.10 - 16.30 COFFEE AND TEA BREAK

16.30 – 16.55 **Nobuhiro KURABAYASHI**
DYRK1A overexpression contributes to enhanced astrogliogenesis in a Down syndrome mouse model

16.55 – 17.20 **Aline DUBOS**
Role of Dyrk1a in GABAergic neurons during development

SESSION 5. DYRK1A mutations, dosage & disease — Chair: Masatoshi HAGIWARA

17.20 – 17.45 **Bregje WM VAN BON**
De novo mutations of DYRK1A lead to a syndromic form of ID



17.45 – 18.10 **Amélie PITON**
DYRK1A haploinsuficiency is a frequent cause of intellectual disability: How to better diagnose it?

18.10 – 18.35 **Véronique BRAULT**
Dyrk1a gene dosage in glutamatergic neurons has a key effect in cognitive deficits observed in mouse models of MRD7 and Down syndrome

THURSDAY MARCH 30 – morning

SESSION 6. DYRK & CLK inhibitors — Chair: Stefan KNAPP

9.00 – 9.25 **Masatoshi HAGIWARA**
Development of inhibitors of CDK9, CLK1, DYRK1a, and their clinical application

9.25 – 9.50 **Thierry BESSON**
New DYRK1A inhibitors

9.50 – 10.15 **Franz BRACHER**
Novel DYRK1 inhibitors derived from β-carboline alkaloids

10.15 – 10.40 **Robin KETTELER**
Novel Scaffold Kinase Inhibitors of DYRK/CLK Family Members

10.40 - 11.00 COFFEE AND TEA BREAK

11.00 – 11.25 **Stefan KNAPP**
Rational design strategies for improving selectivity of inhibitors targeting splicing regulating kinases

11.25 – 11.50 **Conrad KUNICK**
DYRK and CLK inhibitors: Is selectivity feasible?

11.50 – 12.15 **Rosanna MEINE**
2-Substituted indole-3-carbonitriles as new DYRK inhibitors

12.15 – 12.40 **Laurent MEIJER**
Leucettines, a family of DYRK1A inhibitors: from marine sponge to drug candidate

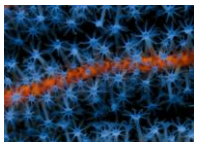
12.40 -14.30 LUNCH

THURSDAY MARCH 30 – afternoon

14.30 – 14.55 **Jonathan C. MORRIS**
Developing inhibitors of the kinases that regulate alternative splicing

14.55 – 15.20 **Scott HENDERSON**
Making the most of public domain data with Knime® : renovating GSK3β and CDK2 scaffolds

15.20 – 15.30 **Marie-Louise JUNG**
Smart screening libraries and successful medicinal chemistry: kinase inhibitors search



SESSION 7. DYRK1A & Diabetes — Chair: Conrad KUNICK

15.30 – 15.55 **Bridget K. WAGNER**
Inhibition of DYRK1A in the pancreatic beta cell

15.55 – 16.20 **Robert J. DE VITA**
DYRK1A as a small molecule target for human *β*-cell proliferation for the treatment of diabetes

16.20 – 16.40 COFFEE AND TEA BREAK

SESSION 8. DYRK1B — Chair: Simon COOK

16.40 – 17.05 **Walter BECKER**
Impaired maturation of DYRK1B mutants that are associated with a form of the metabolic syndrome

17.05 – 17.30 **Maria GAITANOU**
Mirk/Dyrk1B kinase is a novel dual function molecule inducing cell cycle exit and neuronal differentiation in the embryonic chick spinal cord

17.30 – 17.55 **Edward P. GELMANN**
Blocking DYRK1B Phosphorylation of NKX3.1 Prostate Tumor Suppressor Inhibits Prostate Growth and Increases Apoptosis – A Potential Therapeutic Strategy.

17.55 – 18.20 **Rachael HUNTLY**
Analysis of DYRK signalling by phospho-SILAC mass spectrometry

FRIDAY MARCH 31 – morning

SESSION 9. DYRKs, DNA repair and cancer — Chair: Marc BLONDEL

9.15 – 9.25 **Martin MEHNERT**
Multilayered proteomic analysis of cancer mutations in the Dyrk2 kinase complex

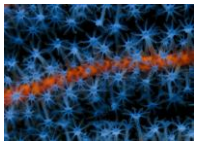
9.25 – 9.50 **Kiyotsugu YOSHIDA**
Tumor suppressive function of DYRK2

9.50 – 10.15 **Julia ROEWENSTRUNK**
Interaction of the protein kinase DYRK1A with RNF169 suggests a role for this kinase in DNA repair

10.15 – 10.40 **Larisa LITOVCHICK**
The role of DYRK1A in DNA repair

10.40 – 11.00 COFFEE AND TEA BREAK

11.00 – 11.25 **Athena F. PHOA**
Drug-target engagement and efficacy of DYRK1A inhibitors in glioblastoma cells



11.25 – 11.50 **Rahul BHANSALI**
The multi-faceted regulatory role of DYRK1A in normal and malignant lymphopoiesis

11.50 – 12.15 **Susana DE LA LUNA**
The DYRK1A kinase positively regulates angiogenic responses in endothelial cells

12.15 – 12.40 **Rajeev SINGH**
DYRK1B and Hedgehog signaling: a complex crosstalk

12.50 - 14.30 LUNCH

FRIDAY MARCH 31 – afternoon

SESSION 10. DYRK1A & T cell regulation — Chair: Laurent MEIJER

14.30 – 14.55 **Bernard KHOR**
From kinome to DYRK1A in T cell regulation and inflammation

SESSION 11. DYRKs substrates & regulators — Chair: Walter BECKER

14.55 – 15.20 **Kassandra M. ORI-MCKENNEY**
Multimodal regulation of the microtubule cytoskeleton by DYRK1a

15.20 – 15.45 **Reinhard W. KOSTER**
Genetic modeling of neurodegenerative diseases in zebrafish for bioimaging studies and compound evaluation

15.45 – 16.10 **Maribel LARA-CHICA**
Identification of new DYRK2 substrates and their implications in carcinogenesis

16.10 – 16.30 COFFEE AND TEA BREAK

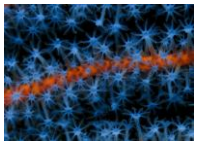
16.30 – 16.55 **Simon COOK**
Analysis of DYRK signalling by RNAseq profiling

16.55 – 17.20 **Yoshihiko MIYATA**
Functional regulation of different DYRK family protein kinases by distinctive cellular binding partners

17.20 – 17.45 **Chiara DI VONA**
DYRK1A as a gene-specific RNA polymerase II CTD kinase

17.45 – 18.10 **Despina SMIRLIS**
Leishmania infantum DYRK1: a negative regulator of the G1 to S cell-cycle transition, essential for the development of infective stationary phase promastigotes

SESSION 12. General conclusions — Chairs: L. MEIJER, C. KUNICK & Y. HERAULT



SATURDAY APRIL 1st - morning

Saturday morning we are meeting with parents of children with DYRK1A mutations (we had a large number of requests, it was rather unexpected, but we thought we had to do something!). This session will be in French. Anyone is welcome, just indicate to Pauline that you would like to join this extra session.

SESSION 13. Session grand public: Présentation de la protéine DYRK1A, ses fonctions, ses régulateurs et son implication dans diverses pathologies humaines : retour sur les résultats présentés lors du congrès **Chair: Amélie PITON**

10.00 – 10.30 Yann **HERAULT** & Laurent **MEIJER**
Résumé de la conférence, points forts des avancées sur la connaissance de DYRK1A

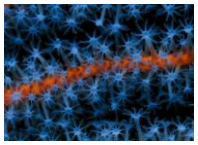
10.30 – 11.00 PAUSE CAFÉ & THÉ

11.00 – 11.30 Amélie **PITON**, Marie **VINCENT**, Marjolaine **WILLEMS**
Le syndrome MRD7

11.30 – 11.45 Yann **HERAULT**
Le modèle souris pour mieux comprendre le syndrome MRD7

11.45 – 12.30
Paroles aux familles, discussion

12.30 LUNCH



ABSTRACTS

(by alphabetical order of speaker)

Control of neuron numbers by DYRK1A: lessons from mouse models

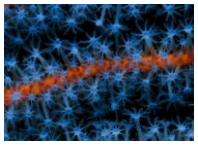
Sònia NAJAS, María JOSE BARALLORE, Mariona L. ARBONES

Instituto de Biología Molecular de Barcelona (IBMB-CSIC), c/ Baldiri i Reixac 15, 08028 Barcelona, Spain

Neurons in the mammalian brain are generated prenatally from a heterogeneous population of progenitors that divide producing more progenitors (expansion divisions) or producing neurons (differentiative divisions). Neurons are usually generated in excess and a fraction of them die during development by physiological apoptosis. Therefore, alterations in the neurogenic potential of embryonic neural stem cells or in the activity of apoptotic cell death pathways may have a significant impact in the number of the different neuron types that integrate into functional circuits. Studies in mouse models carrying 1 or 3 functional copies of *Dyrk1a* have shown that DYRK1A controls brain size and neuron numbers in a dosage-dependent and region-specific manner (1). In both haploinsufficient *Dyrk1a*$^{+/-}$ embryos and transgenic embryos carrying 3 copies of mouse *Dyrk1a* (TgBAC*Dyrk1a*), neurogenesis is preserved in regions of the ventral mesencephalon where dopaminergic neurons involved in the control of voluntary movement and regulation of emotion are generated. However, at postnatal stages the number of these neurons were decreased in *Dyrk1a*$^{+/-}$ mice and increased in TgBAC*Dyrk1a* mice due to a dysregulation of Caspase 9-mediated cell death pathway (2). In contrast, neuron counts in the postnatal neocortex of *Dyrk1a* mutant mice, the region involved in higher-order brain functions, inversely correlate with DYRK1A protein levels. Examination of this structure indicated that neurogenesis is increased in the *Dyrk1a*$^{+/-}$ model and reduced in the TgBAC*Dyrk1a* model, and that variations in the division mode (proliferative *vs.* differentiative divisions) of the stem cells (radial glial progenitors) that give rise to cortical excitatory neurons contribute to the neurogenic defects observed in these two models. Reduced neurogenesis in TgBAC*Dyrk1a* embryos correlates with a longer cell cycle G1 phase and decreased nuclear levels of the cell cycle activator Cyclin D1 in radial glial progenitors. These defects are consistent with the ability of DYRK1A to phosphorylate T286 in Cyclin D1, which promotes its nuclear export and subsequent degradation *via* the ubiquitin-proteasome pathway (3). During the talk, I will present new data showing the effect of DYRK1A overexpression in the production of cortical inhibitory neurons and discuss the pathogenic effects of DYRK1A gene-dosage variations in neocortical development.

(1) Guedj et al., 2012. **Neurobiol. Dis. 46**, 190-203.
(2) Barallobre et al., 2014. **Cell Death Dis. 5**, e1289.
(3) Najas et al., 2015. **EBioMedicine 2**, 120-134.

Financial support: this work was supported by the Spanish Ministry of Economy, Innovation and Competitiveness (MINECO), the Spanish network on Rare Diseases (CIBERER) and the Jérôme Lejeune Foundation.



Impaired maturation of DYRK1B mutants that are associated with a form of the metabolic syndrome

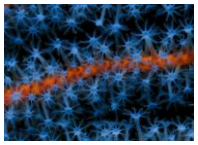
Samira ABU JHAISHA, Esti W. WIDOWATI, Stefan KNAPP[1], Walter BECKER[2]

[1]Institute for Pharmaceutical Chemistry and Buchmann Institute for Molecular Life Sciences (BMLS), Johann Wolfgang Goethe University, Frankfurt am Main, Germany. [2]Institute of Pharmacology and Toxicology, RWTH Aachen University, Aachen, Germany

DYRK family protein kinases depend on the autophosphorylation of a conserved tyrosine residue in the catalytic domain to acquire full catalytic activity (1). In addition to the sequence similarity of their kinase domains, all DYRK kinases share a structural element named DYRK homology (DH) box in the N-terminal domain (2). Recently, two missense mutations affecting the DH box of DYRK1B have been found to co-segregate with a rare autosomal-dominant form of the metabolic syndrome (3). The present study aims to elucidate the consequences of these amino acid substitutions (H90P and R102C) on the molecular function of DYRK1B.

In vitro kinase assays showed that the mutations did not alter the specific activity of mature kinase molecules. However, cell culture experiments showed that a significant part of the mutant DYRK1B protein accumulated in detergent-insoluble cytoplasmic aggregates and was underphosphorylated on tyrosine. The mutant DYRK1B variants were more vulnerable to the HSP90 inhibitor ganetespib and showed enhanced binding to the co-chaperone CDC37 as compared to wild type DYRK1B. These results support the hypothesis that the mutations in the DH box interfere with the maturation of DYRK1B by tyrosine autophosphorylation and compromise the conformational stability of the catalytic domain, which renders the kinase susceptible to misfolding and aggregation.

(1) Becker W & Sippl W, 2011. Activation, regulation, and inhibition of DYRK1A. **FEBS J. 278**, 246-56.
(2) Becker W & Joost HG, 1999. Structural and functional characteristics of Dyrk, a novel subfamily of protein kinases with dual specificity. **Progr. Nucleic Acids Res. Mol. Biol. 62**, 1-17.
(3) Keramati AR et al., 2014. A form of the metabolic syndrome associated with mutations in DYRK1B. **N. Engl. J. Med. 370**, 1909-1919.

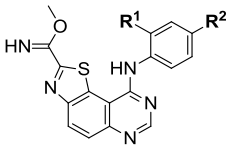


New DYRK1A inhibitors

Thierry BESSON

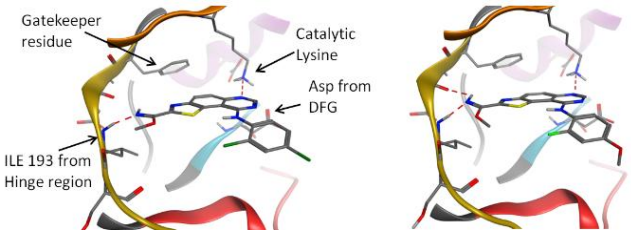
Normandie University, UNIROUEN, INSA Rouen, CNRS, COBRA UMR 6014, 76000 Rouen, France.

Our research group is mainly invested in the synthesis of C,N,S- or C,N,O-containing heterocyclic precursors of bioactive molecules able to modulate the activity of kinases in signal transduction, and especially Ser/Thr kinases (CDK5, GSK3, CLK1 and CK1) and dual-specificity kinases (DYRK family). DYRK1A is certainly the most studied and is a novel, high-potential therapeutic target for pharmacological interventions seeking to modify the course of Alzheimer's disease. In our continuous effort aiming at preparing novel heterocyclic scaffolds able to modulate the activity of kinases synthetic routes to functionalized thiazolo[5,4-*f*]quinazolines were particularly studied The chemical highlight of this work was the use of a special reagent (4,5-dichloro-1,2,3-dithiazolium chloride) and a 6-amino-2-cyanobenzo[*d*] thiazole-7-carbonitrile derivatives as a versatile molecular platform. Introduction of various aliphatic, aromatic or amino substituents and transformation of carbonitrile group into various chemical functions (*e.g.* imidate, ester, amidine...) allowed the efficient preparation of a library of novel thiazoloquinazoline derivatives. The most active compounds are five methyl carbimidate derivatives exhibiting significant and selective inhibition against DYRK1A and DYRK1B (subnanomolar range).



Compound	R^1	R^2	DYRK1A / 1B*
1 (EHT 5372)	Cl	Cl	0.22 / 0.28
2 (EHT 1610)	F	OMe	0.36 / 0.59
3 (EHT 9851)	F	F	0.94 / 1.07
4 (EHT 6840)	F	Cl	0.99 / 1.63
5 (EHT 3556)	H	Me	0.98 / 2.83
Harmine			21.83 / 57.4
Leucettine L41			7.60 / 37.0

* IC_{50} (nM)

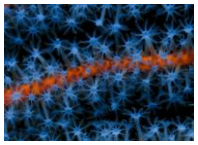


Figures. (**Left**) Chemical structures of methyl 9-anilinothiazolo[5,4-*f*]quinazoline-2-carbimidates **1**-**5** and DYRK1A and DYRK1B IC_{50} (nM) values. (**Right**) Modelling of **1** and **2** with DYRK1A/1B, calculated from structural modelling and docking studies.

The crystal structures of the complex revealed a non-canonical binding mode of compound **1** and **2** in DYRK2 explaining the remarkable selectivity and potency of these inhibitors. The structural data and comparison presented here provide therefore a template for further improvement of this inhibitor class and for the development of novel inhibitors selectively targeting DYRK kinases.

(1) Leblond,B. *et al., 2013.* WO 2013026806. **Chem. Abstr. *158***, 390018.
(2) Foucourt A., *et al.*, 2014. **Molecules 19**, 15546.
(3) Foucourt A., *et al.*, 2014. **Molecules 19**, 15411.
(4) Thompson, B., *et al., 2015.* ***J. Exp. Med.*** **212**, 723.
(5) Courtadeur, S., *et al., 2015.* **J. Neurochem. 133**, 440.
(6) Besson, T. *et al., 2016.* **J. Med. Chem. 59**, 10315.

Financial support : financial support from the MESR (Ministère de l'Enseignement Supérieur et de la Recherche) is gratefully acknowledged. We also thank the LABEX SynOrg (ANR-11-LABX-0029) for financial support.



The multi-faceted regulatory role of DYRK1A in normal and malignant lymphopoiesis

Rahul BHANSALI[1], Paul LEE[2], Malini RAMMOHAN[1], John CRISPINO[1]

[1]Division of Hematology/Oncology Northwestern University, Chicago, IL, USA; [2]Division of Pediatric Hematology/Oncology, Ann and Robert H. Lurie Children's Hospital, Chicago, IL, USA

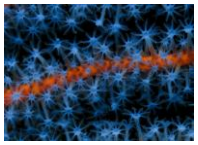
The chromosome 21 gene *DYRK1A* encodes a versatile kinase implicated in various roles integral to cellular function. Our group found a tumorigenic role for *DYRK1A* in Down syndrome-Acute Megakaryoblastic Leukemia (1), and we subsequently studied its role in normal hematopoiesis using a conditional knockout mouse model. Ultimately, we uncovered that *DYRK1A* is required for normal lymphoid, but not myeloid, development through its phosphorylation and destabilization of cyclinD3 leading to quiescence and cellular maturation. While loss of cell cycle exit primarily stems from stabilization of cyclinD3, these cells do not proliferate uncontrollably; rather, they show premature exhaustion (2). This finding prompted our proteomics-based investigation into the various roles of DYRK1A in lymphopoiesis and its possible involvement in the pathogenesis of Acute Lymphoblastic Leukemia (ALL).

In order to elucidate which pathways were affected by DYRK1A inhibition, we treated primary murine pre-B cells with a DYRK1-specific inhibitor, followed by tandem mass tagging and analysis by mass spectrometry. This generated several thousand peptides that were differentially phosphorylated between the treated and untreated samples. With an inclusion criterion of 1.5-fold decrease in phosphorylation upon DYRK1A inhibition, we refined our list to 36 proteins, including cyclin D3, which are strong candidates for DYRK1A substrates or members of proximally involved pathways. Using bioinformatics analysis of GO Biological Processes, we found that these proteins showed enrichment of pathways vital for cell development such as cell cycle, cell division and mitosis, RNA metabolism, and JAK-STAT signaling. Further investigation of these 36 proteins has provided an excellent opportunity to develop a more profound understanding of several lymphopoietic pathways that may involve DYRK1A and how these relate to the development of ALL when dysregulated.

Clinically, these results, in combination with data showing that *DYRK1A* expression is increased in ALL relative to other tumor types, implicate DYRK1A as a potential target in ALL. But this study also provides an interesting perspective in cancer biology because this versatile kinase may have both oncogene and tumor suppressor qualities in the same cell type, elaborating upon the traditional models of carcinogenesis.

(1) Malinge, S., et al., 2012. Increased dosage of the chromosome 21 ortholog Dyrk1a promotes megakaryoblastic leukemia in a murine model of Down syndrome. **J. Clin. Invest. 122**, 948-962.
(2) Thompson, B.J., et al., 2015. DYRK1A controls the transition from proliferation to quiescence during lymphoid development by destabilizing Cyclin D3. **J. Exp. Med. 212**, 953-970.

Financial support: we would like to thank the Rally Foundation for Childhood Cancer Research and the American Society of Hematology for their generous support of this project.



Novel DYRK1 inhibitors derived from β-carboline alkaloids

Franz BRACHER

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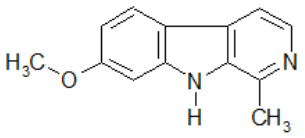
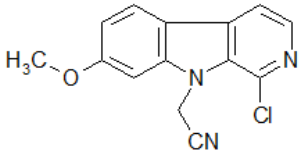
Natural products are a rich source of lead structures for drug development. In the last decade we performed extensive investigations on the potential of β-carboline alkaloids and synthetic analogues thereof as lead structures for the development of selective kinase inhibitors. Accompanied by comprehensive research on novel synthetic approaches towards the β-carboline scaffold, we developed new and selective kinase inhibitors derived from the alkaloids bauerine C (an 1-oxo-β-carboline from a blue-green alga), annomontine (a aminopyrimidyl-β-carboline alkaloid from tropical plants), and the plant alkaloid harmine.

Highly selective inhibitors of DYRK1 were obtained by systematic structure variation of harmine, and the most advanced new inhibitors, e.g. **AnnH75**, were free from undesired MAO A inhibitory activity. Concise structure-activity relationships could be elaborated (in cooperation with Prof. Dr. Walter Becker, Aachen), and a co-crystal structure (PDB: 4YU2; provided by the group of Prof. Dr. Stefan Knapp, Oxford) gave further insight into the binding mode.



harmine



AnnH75

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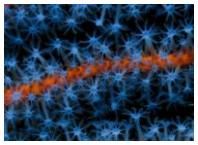

APPβ processing initiates full Tau pathology in a novel age dependent Alzheimer's disease rat model: GSK3β and DYRK1A levels during human-like AD progression

Mickael AUDRAIN, Benoit SOUCHET, Sandro ALVES, Romain FOL, Alexis HADDJERI, Satoru TADA, Charlène JOSEPHINE, Alexis-Pierre BEMELMANS, Nicole DEGLON, Philippe HANTRAYE, Yvette AKWA, Jean-Marie BILLARD, Brigitte POTIER, Patrick DUTAR, Nathalie CARTIER, Jérôme BRAUDEAU[1]

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Alzheimer's disease (AD), the most common form of dementia, is characterized by a progressive accumulation of β-amyloid peptide (Aβ), a gradual Tau hyperphosphorylation, and displays a decline in cognitive functions followed by senile plaques and tangles formation. Despite billions of dollars invested in R&D to find an effective treatment, AD clinical trials still have one of the highest failure rate of any disease area – over 99% compared with 81% for cancer (1). This high failures rate could be attributed in part to current animal models, which do not fully recapitulate the human AD course. Especially, the link between APP processing and Tau pathology remains challenging in transgenic animals.

Indeed, growing evidences suggests that APP processing and soluble amyloid-β (Aβ) release are upstream of Tau pathology. However, the lack of animal models mimicking these both cerebral pathologies as observed in human AD raises questions regarding both amyloid cascade hypothesis validity and underlying mechanism.

In order to decipher relationship between amyloid and tau pathologies, we developed the first inducible and progressive AD rat model, named AAV-AD rat. Here, we induced rat model thanks AAV gene transfer of mutated form of human APP and PS1. This modeling strategy, already described in mouse (2), led to produce amyloid derivatives while avoiding significant transgene overexpression. Soluble Aβ42 levels and the Aβ42/40 ratio gradually increased in rat hippocampus and CSF to levels close to those found in human AD. Senile plaque formation progressively occurred late in the life of the animals, i.e. 2.5 years after induction. More importantly, endogenous Tau was progressively hyperphosphorylated over time and associated with increased levels of GSK3β and DYRK1A kinases, resulting finally in tangle-like aggregation (AT8 positive cells) in old rats.

Detailed analysis of biochemical, histological, electrophysiological, and behavioural critical preclinical steps of AD progression in the AAV-AD rat allowed us to decipher the early links between APP processing and Tau pathology and propose a sequential AD progression hypothesis during infraclinical stages.

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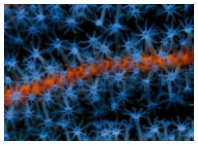

Dyrk1a gene dosage in glutamatergic neurons has a key effect in cognitive deficits observed in mouse models of MRD7 and Down syndrome.

Véronique BRAULT[1,2,3,4], Javier FLORES-GUTIERREZ[1,2,3,4], Marie-Christine BIRLING[5], Guillaume PAVLOVIC[5], Loïc LINDNER[5], Mohammed SELLOUM[5], Tania SORG[5], Valérie LALANNE[5], Hamid MEZIANE[5], Giovanni IACONO[6], Doulaye DEMBELE[1,2,3,4], Yann HERAULT[1,2,3,4,5]

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Perturbation of the excitation/inhibition (E/I) balance leads to neurodevelopmental diseases associated to autism spectrum disorders, intellectual disability and epilepsy. The *DYRK1A* gene present on human chromosome 21 (Hsa21) codes for a kinase with numerous substrates. Mutations in this gene leads to an intellectual disability syndrome associated with microcephaly, epilepsy and autistic troubles (MRD7) (1). In mice, haploinsufficiency of *Dyrk1a* leads to decreased brain size with altered microarchitecture of pyramidal cells (2). Overexpression of *DYRK1A*, on the other hand, has been linked with learning and memory defects observed in people with Down syndrome (DS). Analysis of DS trisomic mouse models revealed that Dyrk1a plays a crucial role in the perturbation of the E/I balance that leads to the deficit in synaptic transmission and memory observed in those models (3).Those findings led to preclinical programs using either GABA antagonists or DYRK1A inhibitors to rescue learning and memory deficits in DS mouse models. Dyrk1a is expressed in both glutamatergic and GABAergic neurons, but its impact on each neuronal population has not yet been elucidated. We started to investigate the impact of *Dyrk1a* gene copy number variation in glutamatergic neurons using a genetic approach. We used a conditional knockout allele of *Dyrk1a* mated with a transgenic mouse expressing the Cre recombinase in hippocampal and cortical glutamatergic neurons (Tg(Camk1a-Cre)). We combined those mice with the trisomic mouse model Ts1Yey to return to two copies of *Dyrk1a* in glutamatergic neurons. We also produced *Dyrk1a*$^{Camk2a/+}$ and *Dyrk1a*$^{Camk2a/Camk2a}$ mice in order to look at the impact of the deficit of *Dyrk1a* in glutamatergic neurons. *Dyrk1a* gene dosage change in glutamatergic neurons did not impact working memory deficits or susceptibility to epilepsy as tested with the pro-convulsivant PTZ. However, object recognition memory was impacted by gene copy number indicating a major effect of *Dyrk1a* trisomy on the glutamatergic pathway in declarative memory.

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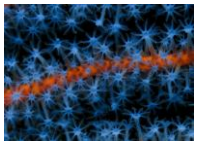
Financial support: the project is supported by the French National Centre for Scientific Research (CNRS), the French National Institute of Health and Medical Research (INSERM), the University of Strasbourg and the “Fondation Jerôme Lejeune”.



A chemical with proven clinical safety rescues Down-syndrome-related phenotypes through DYRK1A inhibition

Miri CHOI[1,8], Hyeongki KIM[1,2], Kyu-Sun LEE[3,4], Ae-Kyeong KIM[3], Kwangman CHOI[1], Mingu KANG[1,8], Seung-Wook CHI[5], Min-Sung LEE[5], Jeong-Soo LEE[3,4], So-Young LEE[6], Woo-Joo SONG[7], Kweon YU[3,4], and Sungchan CHO[1,2]

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DYRK1A is important in neuronal development and function, and its excessive activity is considered a significant pathogenic factor in Down syndrome and Alzheimer's disease. Although the inhibition of DYRK1A is a new strategy to modify the disease, very few inhibitors have been reported yet, and their potential clinical uses require further evaluation. Here, we newly identify CX-4945, the safety of which has been already proven in the clinical setting, as a potent inhibitor of DYRK1A that acts in an ATP-competitive manner. The inhibitory potency of CX-4945 on DYRK1A (IC_{50}=6.8 nM) in vitro was higher than that of harmine, INDY or proINDY, which are well-known potent inhibitors of DYRK1A. CX-4945 effectively reverses the aberrant phosphorylation of Tau, amyloid precursor protein (APP) and presenilin 1 (PS1) in mammalian cells. To our surprise, feeding with CX-4945 significantly restored the neurological and phenotypic defects induced by the overexpression of minibrain, an ortholog of human DYRK1A, in the Drosophila model. Moreover, oral administration of CX-4945 acutely suppressed Tau hyperphosphorylation in the hippocampus of DYRK1A-overexpressing mice. Our research results demonstrate that CX-4945 is a potent DYRK1A inhibitor and also suggest that it has therapeutic potential for DYRK1A-associated diseases. Currently, to determine whether CX-4945 rescues the cognitive deficits in DS and AD, the behavioral tests in DYRK1A-overexpressing mouse model is on the way.

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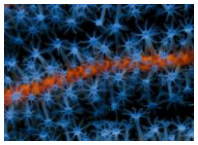

Preclinical study of safety and biomarkers of efficacy of Epigallocatechin-3-gallate at three different doses in wild-type and transgenic DYRK1A mice.

Cecile CIEUTA-WALTI[1,2], A.-S. REBILLAT[1], J.M. DELABAR[3], J.L. PAUL[4], J. DAIROU[5], N. JANEL[6].

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Objective : To study the safety and biomarkers of efficacy of three doses of Epigallocatechin-3-gallate (EGCG) in wild-type and TgDYRK1A mice for the purpose of conducting a clinical study in children with Down Syndrome.

Methods: The group 1 consisting of 4 subgroups of 20 wild-type (WT) mice who were fed with EGCG (Fontup) for 3 times at dose 1 (25 mg/kg), 2 (50 mg/kg), 3 (75 mg/kg) and 4 (placebo). The group 2 of WT and transgenic (Tg)DYRK1A mice were fed with dose 3 twice a day (150 mg/kg/d) for one week. Blood analyses of plasma efficacy (plasma EGCG dosage, homocysteine, GSH, fibrinogen, ApoD) have been down in the group 1 and safety and neural markers of efficacy (plasma DYRK1A, ALT, galectin-3, brain BDNF, P-ERK) in groups 1 and 2.

Results: blood analyses of efficacy show in the group 1: plasma level of EGCG is proportional to the administered dose ($p<0.0001$). Plasma homocysteine level is only significantly increased ($p<0.01$) with the dose 2 with a non-pathologic rate. Plasma GSH level is increased for dose 2 ($p<0.03$) and 3 ($p<0.001$). Plasma level of fibrinogen is decreased ($p<0.0001$) at doses 1, 2 and 3. Plasma ApoD level is decreased ($p<0.02$) for dose 2 and 3 and for dose 1 ($p<0.005$); blood analyses of safety show in the group1: plasma DYRK1A level is unchanged. Plasma ALT level decreased with dose 2 ($p<0.07$) and appears to have a protective effect on the liver. Plasma level of galectin-3 (a marker of cardiac function) is unchanged; blood analyses of safety show in the group 2 (150 mg/kg/d for one week): plasma ALT level is not significantly increased in TgDYRK1A mice and unchanged in WT mice. Plasma level of Galectin-3 is decreased in the basal condition in TgDYRK1A mice ($p<0.01$) and is not significantly increased with dose 3. Plasma level of galectin-3 decreased in WT mice ($p<0.001$); blood analyses of neural markers of efficacy show for the group 1: brain BDNF level is increased respectively for dose 1 and 3 ($p<0.0001$) and for dose 2 ($p<0.05$). Brain PERK/ERK level is decreased for the dose 1 ($p<0.0001$), 2 ($p<0.06$) and 3 ($p<0.03$). Additional analyses were done with dose 2 in brain TgDYRK1A mice and showed that two neural markers of efficacy, PERK/ERK ($p<0.01$) and DDB1 ($p<0.03$) levels are decreased.

Conclusion: This preclinical study shows that EGCG is safe for hepatic and cardiac function at these 3 doses in WT mice, which correspond in humans approximately to 2.5, 5 and 7.5 mg/kg and with the dose of 15 mg/kg/d during one week. Blood analyses show that plasma level of EGCG, homocysteine, GSH, ApoD are good biomarkers of EGCG efficacy and that dose 2 is probably the best dose, which corresponds in humans to 5 mg/kg per dose, i.e. 10 mg/kg/d. These results confirm the dose to be used for safety and potential efficacy of EGCG in our future clinical study in children with Down Syndrome.

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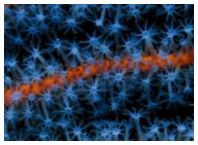

Analysis of DYRK signalling by phospho-SILAC mass spectrometry

Rachael HUNTLY[1], Anne ASHFORD[1], David OXLEY[1], Kathryn S. LILLEY[2] and Simon J. COOK[1].

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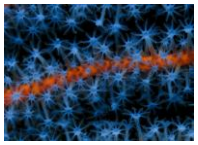
The DYRKs are proline-directed protein kinases that sit in the CMGC arm of the human kinome, being distantly related to the ERKs and CDKs. Hundreds of substrates/interacting partners are known for the ERKs and CDKs and we have a good conceptual understanding of how these protein kinases function. In contrast, relatively few DYRK substrates have been defined that might provide a biological context for DYRK functions. Since the DYRKs undergo co-translational *cis*-autoactivation we have utilised tetracycline-inducible expression systems to drive DYRK1B or DYRK2 expression in HEK293 cells coupled with stable isotope labelling of amino acids in cell culture (SILAC), phosphopeptide enrichment and LC-MS/MS to identify DYRK-inducible phosphoproteins. Mascot and Proteome Discoverer™ software packages allowed identification and quantification of phosphopeptides whilst Motif-x revealed that the majority of DYRK-induced phosphorylation events were at pS-P or pT-P motifs, consistent with them potentially being direct DYRK substrates. Gene Ontology (GO) analysis for both DYRK1B- and DYRK2-induced changes in phosphorylation suggested RNA processing/metabolism, protein turnover, translation, signal transduction and mitosis as DYRK-regulated processes. Follow-up validation of the hits from these screens is ongoing but we have already confirmed the identification of new substrates of DYRK1B and DYRK2 involved in RNA processing and protein turnover/proteostasis.

Financial support: work funded by a Biotechnology and Biological Sciences Research Council (BBSRC) PhD studentship (University of Cambridge), a BBSRC Project Grant and a BBSRC Institute Strategic Programme Grant.



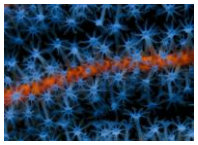
DYRK1A, biomarker or target for Alzheimer's disease

N JANEL, P ALEXOPOULOS, A BADEL, F LAMARI, A-C CAMPROUX, J LAGARDE, S SIMON, C FERAUDET-TARISSE, P LAMOURETTE, M ARBONES, JL PAUL, B DUBOIS, MC POTIER, M SARAZIN, Jean-Maurice DELABAR

Institut du cerveau et de la moelle, CNRS UMR 7225, INSERM U1127, UPMC, 47 bd de l'Hôpital, CS21414, 75646 Paris Cedex, France.

Early identification of Alzheimer's disease (AD) risk factors would aid development of interventions to delay the onset of dementia, but current biomarkers are invasive and/or costly to assess. Validated plasma biomarkers would circumvent these challenges. We previously identified the kinase DYRK1A in plasma. To validate DYRK1A as biomarker for AD risk, we assessed levels of DYRK1A and the related markers BDNF and homocysteine in two unrelated AD patient cohorts with age-matched controls.

Receiver-operating-characteristic curves and logistic regression analyses showed that combined assessment of DYRK1A, BDNF, and homocysteine has a sensitivity of 0.952, a specificity of 0.889, and an accuracy of 0.933 in testing for AD. The blood levels of these markers provide a risk assessment profile. Combined assessment of these three markers outperforms most of the previous markers and could become a useful substitute to the current panel of AD biomarkers. These results associate a decreased level of DYRK1A with AD and challenge the use of DYRK1A inhibitors in peripheral tissues as treatment. It may also help to predict future cognitive decline in cognitively normal individuals.

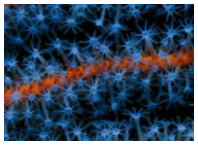


The DYRK1A kinase positively regulates angiogenic responses in endothelial cells

Esteban J. ROZEN[1], Julia ROEWENSTRUNK[1], María J. BARALLOBRE[2], Chiara DI VONA[1], Carole JUNQ[3], Ana F. FIGUEIREDO[4], Jeroni LUNA[5], Cristina FILLAT[5], Mariona L. ARBONES[2], Mariona GRAUPERA[4], Miguel A. VALVERDE[3], Susana DE LA LUNA[1]

[1]Gene Regulation, Stem Cells and Cancer Programme, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain. [2]Institut de Biologia Molecular de Barcelona (IBMB), Barcelona, Spain. [3]Molecular Physiology Laboratory, Universitat Pompeu Fabra (UPF), Barcelona, Spain. [4]Vascular Signalling Laboratory, Institut d´Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, Spain. [5]Institut D'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

Angiogenesis is a highly regulated process essential for correct organ development and maintenance, and its deregulation contributes to inflammation, cardiac disorders and cancer. The Ca^{2+}/calcineurin/Nuclear Factor of Activated T-cells (NFAT) signaling pathway is central to endothelial cell angiogenic responses, and it is activated by stimuli like the vascular endothelial growth factor (VEGF). NFAT activity is regulated by phosphorylation/dephosphorylation, and phosphorylation by dual-specificity tyrosine phosphorylation-regulated kinases (DYRKs) is thought to be an inactivating event. Contrary to the expectations, we show here that the DYRK family member DYRK1A positively regulates NFAT transcriptional responses in primary endothelial cells following VEGF stimulation. Down regulation of DYRK1A in endothelial cells reduces the release of Ca^{2+} from intracellular stores in response to VEGF, which results in increased NFAT phosphorylation, increased cytoplasmic NFAT accumulation and consequently, reduced NFAT-dependent transcriptional responses. The DYRK1A effect appears to be exerted at the level of VEGF receptor accumulation leading to diminished PLCγ1 activation. DYRK1A knockdown markedly impairs VEGF-mediated endothelial cell proliferation and tube formation. Concurring with the *in vitro* results, *Dyrk1a* heterozygous mice show defects in developmental retinal vascularization and in VEGF-mediated vascular outgrowth in aortic ring assays. Therefore, our data establish a novel regulatory circuit, DYRK1A/ Ca^{2+}/NFAT, that is critical to fine-tune endothelial cell proliferation and angiogenesis.



DYRK1A as a Small Molecule Target for Human *β*-Cell Proliferation for the Treatment of Diabetes

K. KUMAR; P. WANG; H. WANG; H. LI; P.UNG; A. SCHLESSINGER; DP. FELSENFELD; H. LIU; S.SIVENDRAN; A. BENDER; A. KUMAR; J.C. ALVAREZ-PEREZ; A. GARCIA-OCANA; R. SANCHEZ; D.K. SCOTT; A.F. STEWART; R.J. DE VITA

Icahn School of Medicine at Mt. Sinai, New York, USA

Dual Specificity Tyrosine-Regulated Kinase 1a (DYRK1A) is a kinase that has been implicated in multiple CNS disease states including Alzheimer's disease, brain development and Down's Syndrome. Recently, we have reported that small molecule kinase inhibitors, harmine and INDY, are able to induce human β-cells to proliferate *in vitro* and *in vivo* (1). We have also defined one key target relevant to human β-cell replication to be DYRK1A, a result confirmed by two other labs (2, 3). This exciting discovery that small molecule compounds with DYRK1A inhibitory activity promote human pancreatic β-cell proliferation provides a unique opportunity for potential translation to human clinical studies to treat the β-cell deficiency that characterizes all types of diabetes.

We will present details on the validation of DYRK1A as one target important for β-cell proliferation. With that target in hand, the translational effort shifts to questions regarding the optimal kinase selectivity for β-cell proliferation, removing non-kinase off-target activities of known DYRK1a inhibitors and de novo design of new DYRK1a inhibitor scaffolds. We will present our efforts to address these important translational challenges to optimize small molecule DYRK1a inhibitor compounds to treat diabetes and opportunities to deliver them specifically to the beta cell.

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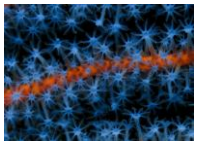

DYRK1A as a gene-specific RNA polymerase II CTD kinase

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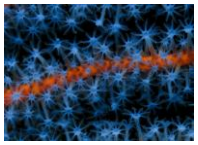
Our perception of how kinases regulate gene expression has recently been expanded to consider not only their influence on transcription factors and co-regulators but also that on histones, chromatin remodelers or components of the basal transcription machinery, all of which may be directly modified by kinases at specific genomic loci. DYRK1A (dual-specificity tyrosine-regulated kinase) belongs to a highly conserved family of kinases represented in all eukaryotes and it is known to fulfil key roles during brain development. We have mapped the genome-wide profile of DYRK1A interactions with chromatin and found that the kinase is recruited to RNA polymerase II (RNAPII)-dependent promoters. DYRK1A binds chromatin regions displaying a highly conserved palindromic sequence that lies close to the transcription start site of target genes, a sequence that appears to be necessary for DYRK1A-mediated transcriptional activation. The recruitment of RNAPII at the promoters of target genes is reduced in cells depleted of DYRK1A, indicating that the reduction in DYRK1A levels could impact negatively on the association of the preinitiation complex with promoters. Moreover, DYRK1A phosphorylates the carboxy-terminal domain (CTD) of the RNAPII both at serine 2 and serine 5 *in vitro*. Consistently, silencing of DYRK1A leads to a reduction in RNAPII phosphorylation in these two residues along the body of targets genes, and to a decrease in the expression of downstream target genes. Interestingly, a subset of DYRK1A targets comprises ribosomal protein genes, and indeed, downregulation of DYRK1A diminishes cell growth. We thus propose a role for DYRK1A as a gene specific CTD kinase and as chromatin-associated transcriptional regulator that is part of the cellular machinery controlling cell growth.



Role of Dyrk1a in GABAergic neurons during development

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Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (*DYRK1A*) is considered to be the major candidate gene for cognitive deficit in Down syndrome (DS) and its mutations is responsible for the autosomal dominant mental retardation 7 syndrome (MRD7), a syndromic form of intellectual disabilities with microcephaly, epilepsy and autistic troubles. In mice, *Dyrk1a* over-expression (*TgDyrk1a*) leads to increased brain size (1) and an increase of GABAergic markers and neurons (2). Strikingly, *TgDyrk1a* mice present an Excitation/Inhibition (E/I) imbalance toward inhibition which confers them resistance to pentylenetetrazol (PTZ)-induced seizures (2). On the other hand, mice heterozygous for *Dyrk1a* present a microcephaly (1, 3), smaller glutamatergic pyramidal cells and decreased GAD67 GABAergic marker (2-4). The overall converge toward increased excitation which lead to increased susceptibility to PTZ-induced seizures in $Dyrk1a^{+/-}$ mice (2). This suggest an essential role of *Dyrk1a* gene dosage in neuronal development and in the establishment of a functional balance between excitatory and inhibitory systems. To determine the pathophysiology mechanisms associated with *Dyrk1a* dosage variations, we analysed the role of *Dyrk1a* during neuronal development by initially focusing on its role on GABAergic neurons development using a *Dyrk1a* conditional KO mouse line crossed with the interneuron specific *Dlx6a-Cre* mouse line.

First, we analysed GABAergic neurons migration at E15.5, period corresponding to a peak in interneuron migration, in $Dyrk1a^{Dlx6a\text{-}Cre/wt}$ mice crossed with the *GAD65-GFP* reported mouse line in order to visualise interneurons. Preliminary results showed a decrease in the total number of GFP+ cells in the neocortex of $Dyrk1a^{Dlx6a\text{-}Cre/wt}$ x *GAD65-GFP* embryos, due to a significant decreased of GFP+ cells in the subventricular zone (SVZ)/intermediate zone (IZ) and the subplate (SP)/cortical plate (CP) migratory streams, but not in the marginal zone (MZ). To better characterise the migration defect observed in $Dyrk1a^{Dlx6a\text{-}Cre/wt}$ x *GAD65-GFP* embryos, we analysed the SVZ/IZ migratory stream at E15.5 and showed a delay of migration of the GFP+ cells in this stream. In addition, the number of GFP+ cells was reduced all along the SVZ/IZ migratory stream. To exclude that the reduction of GFP+ cells was due to defect in interneuron progenitors proliferation, we counted the number of dividing progenitors and did not observed any difference between $Dyrk1a^{Dlx6a\text{-}Cre/wt}$ and $Dyrk1a^{wt/wt}$ embryos, suggesting that it may be due to event occurring after proliferation. Our preliminary results suggest a role of *Dyrk1a* in interneuron development by affecting their migration in the neocortex.

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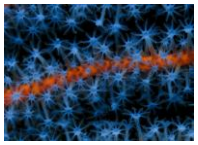

Identification of cystathione β-synthase pharmacological inhibitors to alleviate the intellectual deficiency of patients with Down syndrome

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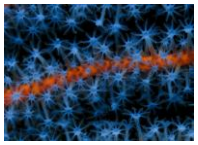
* Both authors contributed equally

Down syndrome (DS) is the most common genetic cause of intellectual disability (ID), affecting 1/700 to 1/1000 live births. Like *DYRK1A*, *CBS* (Cystathionine β-synthase) gene is located on chromosome 21 and both are suspected to be involved in the ID observed in DS. CBS catalyses the condensation of serine and homocysteine to form cystathionine. It plays a critical role by linking the folate and the methionine cycles and by regulating homocysteine levels. In addition, CBS converts cysteine to hydrogen sulfide, an important neuromodulator in the brain. Loss-of-function mutations in *CBS* lead to homocystinuria, characterised by elevated level of homocysteine in urine, skeletal and cardiovascular problems and severe ID. On the contrary, several studies have shown that CBS protein level and enzyme activity are increased in DS patients, suggesting that the level of expression/activity of this enzyme may be critical for proper cerebral function. Accordingly, the group of Y. Hérault has recently observed that *Cbs*-overexpressing transgenic mice present defects in short term memory, suggesting that a partial inhibition of CBS activity may represent a good strategy to improve memory and learning in patients with Down syndrome.

The search for pharmacological inhibitors of CBS has so far been limited to *in vitro* approaches and has not led to the identification of any compounds active *in vivo*. Moreover, no method of chemical library screenings in a eukaryotic cellular context has ever been described. We thus recently developed a yeast model overexpressing CYS4, the orthologue of CBS, and based on the knowledge of the metabolic functions of this enzyme, we set-up a three-steps screening method allowing the identification of molecules specifically inhibiting CYS4/CBS. Using this simple and convenient assay, we screened 2000 FDA-approved drugs. Four different molecules have been found active on both CYS4 and CBS. Interestingly, three of them appear to have common chemical properties. One of them has recently been shown to improve cognitive defects in *Cbs*-overexpressing transgenic mice. As two of these molecules are already in trials or clinics and pass the blood-brain barrier, therapeutic repositioning may be considered at relatively short-term.

Financial support: This work is funded by the Fondation Jérôme Lejeune and has benefited from a seeding grant from the ITMO BCDE (Biologie Cellulaire, Développement et Evolution).



Mirk/Dyrk1B kinase is a novel dual function molecule inducing cell cycle exit and neuronal differentiation in the embryonic chick spinal cord

Nikos KOKKORAKIS[1], Panagiotis K POLITIS[2], Rebecca MATSAS[1] and Maria GAITANOU[1]

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Regulation of cell cycle progression/exit of neuronal precursors is essential for proper generation of the nervous system. Mirk/Dyrk1B has long been studied as a cell cycle regulator in skeletal muscle differentiation controlling cyclin D1 levels by inducing its proteosomal degradation. We have previously demonstrated that Dyrk1B is also expressed in the brain and in primary cortical neurons in culture, while it promotes cell cycle exit and neuronal differentiation in Neuro 2a cells. Here we cloned the chick Dyrk1B ortholog and demonstrated that it is expressed by cycling neuronal progenitors as well as by differentiated motor neurons in the embryonic spinal cord.

Furthermore, we used a *gain-of-function* approach to investigate the role of Dyrk1B *in vivo* in the chick spinal cord. Expression of Dyrk1B/GFP or control GFP protein was achieved by unilateral *in ovo* electroporation in the neural tube of E2 chick embryos, which were then analyzed at E4. We found that overexpression of Dyrk1B induces cell cycle exit. GFP^+ cells in the Dyrk1B/GFP electroporated embryos showed a reduction by 2.3-fold in BrdU incorporation, by 10-fold in the expression of the mitotic marker PH3, by 4.9-fold in Prox-1 and by 1.5-fold in the number of $Sox2^+$ neural progenitors as compared with GFP^+ cells in control embryos. In addition Dyrk1B/GFP electroporated cells showed an increased expression of Doublecortin by 1.4 fold in comparison with GFP electroporated control cells.

In conclusion, we identified Mirk/Dyrk1B as a novel dual function molecule inducing cell cycle exit and neuronal differentiation in the developing chick spinal cord.

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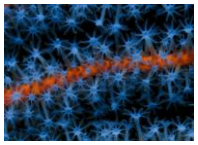

Blocking DYRK1B phosphorylation of NKX3.1 prostate tumor suppressor inhibits prostate growth and increases apoptosis – A potential therapeutic strategy.

Bowen CAI, Lian-Niang SONG, Maria VILENCHIK, Edward P. GELMANN

Columbia University, New York, NY, and Felicitex Therapeutics, Cambridge, MA, USA.

NKX3.1 is a prostate-specific homeodomain protein that is the gatekeeper tumor suppressor of the majority of prostate cancers (1, 2). NKX3.1 is haploinsufficient and mere decrease in protein levels is sufficient to attenuate tumor suppression (3). Moreover, NKX3.1 expression, though reduced in most prostate cancers, is virtually never lost completely, even at sites of advanced, metastatic, castration-resistant prostate cancer (4). As a result, therapeutic intervention designed to increase levels of NKX3.1 protein has the potential to attenuate growth of prostate cancer at any phase of clinical progression. We have shown that DYRK1B phosphorylates NKX3.1 on serine 185 to trigger ubiquitination and mediate steady state protein turnover. Either DYRK1B knock down or its pharmacologic inhibition prolongs NKX3.1 half-life in cultured cells. A third generation DYRK inhibitor, FX9847 that has a very narrow spectrum of kinase inhibition and a high level of activity against DYRK1A, HASPIN/GSG2, and DYRK1B has favorable pharmacokinetics in mice and has been shown to have antitumor activity against pancreatic, lung, and colon cancer xenograft models. In anticipation of testing the effect of FX9847 on the murine prostate, we have shown that FX9847 blocks DYRK1B phosphorylation of NKX3.1 in cultured cells at concentrations that have no adverse effects on cell proliferation. To determine whether *Nkx3.1* gene targeting in the mouse, that causes prostate hyperplasia and dysplasia, is a good model for the effects on the prostate of Dyrk1b inhibition, we engineered mice with mutation of the Dyrk1b target amino acid Nkx3.1(S186), changing the substrate serine to alanine. Mice with only a single copy of this mutant gene, $Nkx3.1^{S186A/-}$, had reduced prostate size, increase Nkx3.1 protein expression, and regions of anoikis and apoptosis in the prostate gland. Next we will cross mice with the $Nkx3.1^{S186A}$ allele with mice engineered for *Pten* loss in the prostate to determine whether Nxk3.1 with prolonged half-life can suppress prostate tumorigenesis activated by *Pten I* loss. These experiments will determine the target phenotype for testing FX9847 in mouse models of prostate cancer.

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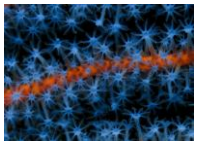
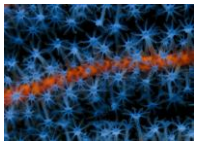


The influence of the metabolism of monocarbons on epigenomic mechanisms: the interacting role of the remethylation and transsulfuration pathways

Jean-Louis GUEANT

Inserm U954, Nutrition-Genetics and Environmental Exposure, Faculty of Medicine, University of Lorraine, 54500 Nancy, France.

The methionine cycle plays a central role in the metabolism of monocarbons. The remethylation of homocysteine catalyzed by methionine synthase in the presence of vitamin B12 and folate allows the synthesis of methionine, which is the immediate precursor of the universal methyl group donor S-adenosylmethionine (SAM). SAM is the substrate of transmethylations reactions involved in epigenomic mechanisms, including the methylation of DNA, RNA and histones, as well as the modulation of the activity of co-regulators of nuclear receptors. Methylation is also involved in the synthesis of intermediate metabolites. The transsulfuration pathway of homocysteine is the cataplerotic pathway of the methionine cycle, which regulates its flux by adapting the concentration of homocysteine. Cystathionine β-synthase (CBS) is the key enzyme of this cataplerotic pathway. The CBS gene is localized on human chromosome 21. CBS removes homocysteine from the methionine cycle and directs the flux of sulfur to the biosynthesis of cysteine. It binds three cofactors, heme, whose function remains an enigma, pyridoxal 5'-phosphate (PLP), part of the catalytic site where homocysteine and serine are condensed to cystathionine, and S-adenosylmethionine (SAM), which upon binding greatly stimulates its activity. The deficiency of CBS results in recessively inherited metabolic disease, homocystinuria, characterized by high concentrations of homocysteine, methionine and S-adenosylhomocysteine and greatly decreased cysteine and cystathionine. As a consequence, deficient CBS activity results in decreased H2S production, which is compensated by enhanced synthesis of hydrogen sulfide. The regulation of the expression and activity of CBS depends on complex regulatory mechanisms that involve the dual-specificity tyrosine-(Y)-phosphorylation regulated kinase (Dyrk1a). The gene of Dyrk1A is also localized on human chromosome 21. Recent data by the group of N. Janel showed that Dyrk1a protein expression is correlated to CBS activity in modified and non-modified genetic mice models, in brain and liver.



Development of inhibitors of CDK9, CLK1, DYRK1a, and their clinical application.

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Kinase families of CDKs, CLKs and DYRKs are involved in the regulation of gene expression. Therefore, we have developed specific inhibitors of these kinases to clarify their physiological functions. Through the development of these chemicals, we eventually succeeded to find candidate compounds available for therapeutic drugs to cure diseases such as viral infections, Duchenne muscular dystrophy, and Down syndrome. The preparation of the clinical trial of the ant-virus drug is under the way in our university hospital as an “Academia Drug”. We propose the strategy to maximize the serendipity for the academic drug discovery with open innovation.

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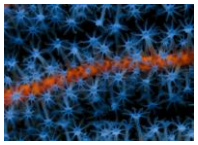

Making the most of public domain data with Knime® : renovating GSK3β and CDK2 scaffolds

Scott HENDERSON[1], James BENNETT[2], Fiona SORRELL[2], Jonathan ELKINS[2], Simon WARD[1]

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A recent publication describes the extensive screening of a set of diverse, drug like kinase inhibitors (Published Kinase Inhibitor Set) and the identification of several new DYRK1A inhibitor templates (1).

Hit-validation and small-scale expansion of a number of these templates has led to the synthesis of inhibitors with nanomolar kinase inhibitory activities against the primary target, DYRK1A. The physicochemical properties of the molecules are close to those required for predicted CNS penetration and drug-likeness (Lipinski Ro5). Encouragingly whilst the compounds have off-target activity (GSK3β and CDK2), making a small change to the structure changes the selectivity profile significantly (2).

The open source Knime® Analytics Platform is being used to predict off-target selectivity, druglikeness central nervous system multiparameter optimization (CNS MPO) score and overall drug likeness in an effort to produce a selective, CNS penetrant tool from which further inhibitors can be derived.

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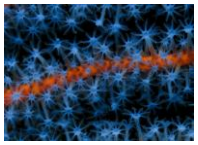

Challenging the role of DYRK1A in Down syndrome by studying the genotype and phenotype relationships in animal models

Yann HERAULT[1,2], Maria DEL MAR MUNIZ MORENO[1], Guillaume PANI[1], Thu Lan NGUYEN[1,3], Damien MARECHAL[1], Claire CHEVALIER[1], Valérie NALESSO[1], Aline DUBOS[1], Michel ROUX[1], Véronique BRAULT[1], Arnaud DUCHON[1]

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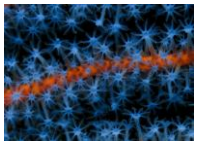
Genetic diseases with intellectual disability (ID) involved impairment of mental abilities that impacts adaptive functioning in the conceptual, the social or the practical domain with or without other features. ID can occur during the developmental period and is defined by an intellectual quotient below 70. Several genetic causes, including trisomy 21 (Down syndrome, DS), deletion or duplication of genomic regions (16p11.2, 17q21.31,…), and more than 700 individual genes, have been associated with ID. To better understand the genotype-phenotype relationship in DS, we generated several models in different organisms. Here we will report the characterization of several DS mouse models using standardized behavioral and cognitive paradigms, completed with transcriptomic approaches in brain region that are affected. Based on the new series of models, several pathways have been identified, challenging the main role of Dyrk1a in DS and highlighting the complexity of genetic interactions. The data generated are challenging our current knowledge on DS and its impact on brain and cognitive functions. Such studies will lead to a better understanding of mechanisms controlling cognition and behavior in model organism and human and how to define new preclinical treatments.



Analysis of DYRK signalling by RNAseq profiling

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Determining 'normal' physiological roles of DYRK1B and DYRK2 is also key to understanding how their deregulation may contribute to disease phenotypes (i.e. cancer and metabolic syndrome). DYRK1B, highly expressed in muscle cells, functions in myoblast differentiation1, but less is known about normal functions of DYRK2. Whilst transcriptional responses to DYRK1B and DYRK2 depletion have previously been explored by microarray analysis (1-3), transcriptional changes that result from their elevated expression are unclear. To investigate DYRK-driven changes on global gene expression, we have utilised tetracycline-inducible expression systems to drive DYRK1B or DYRK2 expression in HEK293 cells followed by cDNA library generation for RNAseq and subsequent data analysis using SeqMonk bioinformatics software. Gene Ontology (GO) analysis for both DYRK1B and DYRK2-induced changes were consistent with our own observations and published work. For example, DYRK1B induction led to differential expression of genes implicated in skeletal muscle differentiation whilst DYRK2 was linked to pro-apoptotic signals. Additionally, GO terms suggested novel functions for DYRK1B in mediating the transcriptional regulation of other major developmental pathways. Finally, our data may provide insights into how the DYRK proteins interact with other known signalling cascades (i.e. MAPK pathways). This data provides novel insights into an understudied area of DYRK biology and highlights clear functional differences at the transcriptional level between class I and class II DYRKs.

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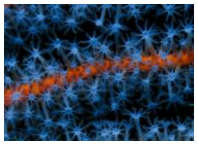

Smart screening libraries and successful medicinal chemistry: kinase inhibitors search

Marie-Louise JUNG

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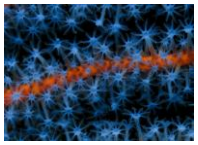
Prestwick Chemical (1), created 1999, has a worldwide recognition for providing smart screening libraries. The Prestwick Drug-Fragment Library is a collection of compounds arising from smart fragmentation of 1565 off-patent approved drugs. The Prestwick CNS Drug Library is a unique collection of 320 structurally diverse approved and marketed central nervous system drugs. The Prestwick Phytochemical Library is a collection of 320 natural products, mostly derived from plants. The *C. Elegans* Library Of Drugs Designed For *Caenorhabditis Elegans* Research Programs is a collection of 240 small molecules that have been carefully selected for chemical structural diversity as well as for good tolerability in *C. elegans*. The Prestwick Pyridazine Library comprises 400 innovative pyridazine and pyridazone derivatives. Finally the most famous chemical library is the Prestwick Chemical Library, a unique collection of 1280 small molecules, mostly approved drugs (FDA, EMA and other agencies) selected by a team of medicinal chemists and pharmacists for their high chemical and pharmacological diversity as well as for their known bioavailability and safety in humans. Over the 16 past years, "hit-likeness" and "hit-workability" of our smart chemical libraries have been substantially reported by users in more than 350 publications. Examples of repositioned drugs into the kinase inhibitors field will be presented. Moreover, an impressive track record of compounds designed and prepared at Prestwick and being in clinical phase can be claimed. Currently 10 molecules are developed in the clinic, coming out of our lead optimization work, and one is on the market. An example for discovering DYRK inhibitors will be emphasized.

(1) http://www.prestwickchemical.com/



Novel scaffold kinase inhibitors of DYRK/CLK family members

Krisna PRAK[1], Janos KRISTON-VIZI[1], A.W. Edith CHAN[2], Christin LUFT[1], Joana R COSTA[1], Niccolo PENGO[1], and Robin KETTELER[1]

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Protein kinases are essential regulators of most cellular processes and are involved in the etiology and progression of multiple diseases. The cdc2-like kinases (CLKs) have been linked to various neuro-degenerative disorders, metabolic regulation, virus infection and autism. Recently, it has been shown that inhibition of CLK2 can restore sociability in a mouse model (1), suggesting that CLK2 is a promising drug target.

Here, we have developed a screening workflow for the identification of potent CLK2 inhibitors and identified compounds with a novel chemical scaffold structure, the benzobisthiazoles that has not been previously reported for kinase inhibitors (2). We propose binding models of these compounds to CLK family proteins and key residues in CLK2 that are important for the compound interactions and the kinase activity. We identified structural elements within the benzobisthiazole that determines CLK2 and CLK3 inhibition, thus providing a rationale for selectivity assays. In summary, our results will inform structure-based design of CLK family inhibitors based on the novel benzobisthiazole scaffold.

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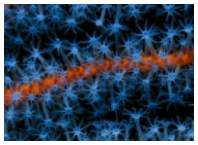

From kinome to DYRK1A in T cell regulation and inflammation

Bernard KHOR, John D GAGNON, Gautam GOEL, Marly I ROCHE, Kara L CONWAY, Leslie N ALDRICH, Thomas B SUNDBERG, Atul K BHAN, John J O'SHEA, Benjamin D MEDOFF, Alykhan F SHAMJI, Stuart L SCHREIBER, Arlene H SHARPE, Stanley Y SHAW, Ramnik J XAVIER

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The immune system balances dueling needs to respond vigorously against pathogens while remaining tolerant to self by engaging pro- and anti-inflammatory forces in precise and dynamic equilibrium. The $CD4^+$ T cell is a central regulator of this equilibrium, acting via its ability to differentiate into either pro-inflammatory effector subsets (e.g. Th1, Th17) or anti-inflammatory subsets, largely represented by regulatory T cells (T_{reg}s). While several key genetic factors regulating these lineage decisions have been identified, therapeutic tools to modulate this balance remain relatively lacking and represent a continuing unmet need.

To this end, we undertook a small molecule discovery approach to identify new, druggable proteins that regulate T cell differentiation. We were particularly interested in kinase targets not only because many facets of T cell biology are known to be regulated by kinases, but also because of the relative advanced state of kinase inhibitor efforts as reflected by their clinical availability, especially in oncology.

In order to capture targets of maximal physiologic relevance, we developed a pipeline to interrogate primary $CD4^+$ T cells in an unbiased high-throughput fashion to identify enhancers of T_{reg} differentiation. We identified DYRK1A as a novel reciprocal regulator of T_{reg}/Th17 differentiation (1). Accordingly, DYRK1A inhibitors exert significant anti-inflammatory effect, promoting T_{reg} while inhibiting Th17 differentiation. These inhibitor-enhanced T_{reg}s appear fully functional in vitro and in animal models of inflammation, and treatment with the DYRK1A inhibitor harmine attenuates inflammation in a murine model of asthma.

These results highlight a potential novel clinical role for DYRK1A inhibition in treating inflammatory disorders. Studies are underway to investigate whether converse DYRK1A hyperactivity provides a unifying explanation for the increased autoimmunity and impaired T_{reg} function in patients with Down syndrome and whether these patients may especially benefit from DYRK1A inhibitor therapies.

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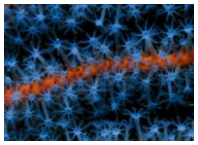

Rational design strategies for improving selectivity of inhibitors targeting splicing regulating kinases

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The CLK, SRPK and DYRK family of kinases have been tightly associated with the regulation of mRNA splicing. Our group is interested in the development of highly selective inhibitors, so called chemical probes, using rational, structure based design approaches targeting each of these kinase families. As a basis for this research we have solved high resolution crystal structures of all CLK family members, DRYK1A as well as SRPK1 and SRPK2 in complex with ATP competitive inhibitors. Structural comparisons and kinome wide inhibitor profiles identified a number of unique structural features that can be used for designing more selective inhibitors. For instance, we recently targeted the unique insertion domain in SRPK1 resulting in the development of inhibitors with exclusive target selectivity. The most selective inhibitor of this series (SPINX31) inhibited SRPK1 at low nM potency *in vitro* as well as in cell based assay systems. In mouse models of macular degeneration, we were able to demonstrate that SRPK1 inhibition leads to a shift in splice form expression from pro-angiogenic to anti-angiogenic VEGF, resulting in potent inhibition of neovascularization in the eye. Some CLK family members harbours unique residue combinations in the ATP site comprising for instance large hydrophobic residues N-terminal to the DFG motif. The presence of these residues result in unique binding modes and a set of typical CLK inhibitor off-targets that share similar residue combination in the active site. However, sequence variations within these kinases allowed us to develop inhibitors with good CLK selectivity. In some cases we were able to obtain also selectivity against the closely related DYRK kinase family.

In this talk I will summarize insights from ligand complexes and how we use them for the development of a selective chemical tool set to study kinase function in mRNA splicing.

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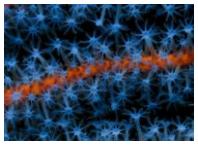
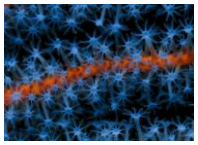


Repression of DYRK1A restores impaired cortical development and abnormal learning behavior of Down syndrome mice

Akiko KOBAYASHI, Isao KII, Yuto SUMIDA, Yukiko OKUNO, Suguru YOSHIDA, Tomonari AWAYA, Takamitsu HOSOYA, Masatoshi HAGIWARA

Department of Anatomy and Developmental Biology, Graduate School of Medicine, Kyoto University, Yoshida-Konoe, Sakyo-ku, Kyoto, 606-8501, Japan

Impaired neurogenesis has been observed in psychiatric/neurodevelopmental diseases such as Down syndrome (DS). Trisomy of chromosome 21, DS, is the most common genetic cause of intellectual disability. Although prenatal diagnosis has become prevalent, no therapies currently exist for the rescue of neurocognitive impairment of DS. Here, we tried a new screen to find out compounds which promote the proliferation of neural stem cells (NSCs). Newly identified growth inducer, which has potent inhibitory activity against dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), rescued proliferative deficits in Ts65Dn-derived neurospheres and human fibroblasts derived from DS individuals. The oral administration of the compound, named ALGERNON, restored the reduced proliferation of NSCs in murine DS models and increased the number of newborn neurons. Moreover, administration of ALGERNON to pregnant dams rescued the abnormal cortical development in DS mouse embryos and abnormal behavior in DS-offspring. These data suggest that DYRK1A represents a potential therapeutic target for individuals with DS, and possibly other disorders involving aberrant neurogenesis.



Genetic modeling of neurodegenerative diseases in zebrafish for bioimaging studies and compound evaluation.

Astrid BUCHBERGER, Kazuhiko NAMIKAWA, Reinhard W. KOSTER

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Zebrafish larvae are small, develop outside their mother, and are produced in large quantities. Moreover, this model organism is genetically tractable with embryos that are nearly transparent and therefore ideally suited for bioimaging studies and compound analysis. We have used these advantages for modelling spinocerebellar Ataxia Type 1 (SCA1), which is caused by a gain of function of nuclear polyglutamine-containing Ataxin 1 (Atx1) resulting in the progressive degeneration of cerebellar Purkinje neurons (PCs) (1). After isolation of a PC-specific regulatory element we have used combinatorial Gal4-genetics to express pathogenic human Atx1 in differentiating zebrafish PCs (2). Aggregate formation and signs of neuronal degeneration can be observed with a few days with Gal4-expression allowing for targeting additional reporters or disease-modulating factors into affected PCs. In addition, these larvae can be easily used to interfere with PC degeneration by compound treatment.

Interestingly, the zebrafish *atx1* homolog together with its evolutionary conserved interaction partners was found to be expressed in sensory hair cells, which become functional already early during development, mediate vibration-induced swimming and are in direct contact with the aqueous medium. Sensory hair cells expressing the human pathogenic Atx1-mutant in transgenic larvae degenerate rapidly resulting in striking behavioural deficits. Furthermore, we show that hair cell degeneration can be rescued either genetically or by small compound treatment providing proof of principle for identifying SCA1-interfering substances that can be tested subsequently for blood-brain-barrier passage.

Zebrafish contain three *dyrk1* homologs. While *dyrk1aa* and *dyrk1ab* have not been studied so far, *dyrk1b* was shown to be expressed during early development and is crucially involved in endoderm formation and craniofacial patterning (3). We have isolated the zebrafish *dyrk1aa* homolog, which is highly conserved to human *dyrk1A*. Expression analysis revealed a nearly pan-neuronal expression during embryonic development. In the adult brain *dyrk1aa* is weakly expressed in the telencephalon, optic tectum and hypothalamus but displays strong expression in the granule cell layer of the cerebellum. A key to genetic modelling of human diseases in zebrafish is the availability of cell type specific regulatory elements. We have set out to establish a bidirectional pan-neuronal enhancer that allows to simultaneously identify transgene expressing cells by fluorescent protein expression. In parallel, a cerebellar granule cell specific regulatory element was identified. This will be used to express human *dyrk1a* throughout the brain or selected neuronal cell types. As proof of principle, we have targeted cerebellar Purkinje cells with human *dyrk1a* overexpression. Transgenic zebrafish do not show gross cerebellar malformations, but appear to display hypoactive behaviour – a phenotype that can be subjected to small compound analysis.

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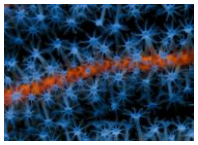
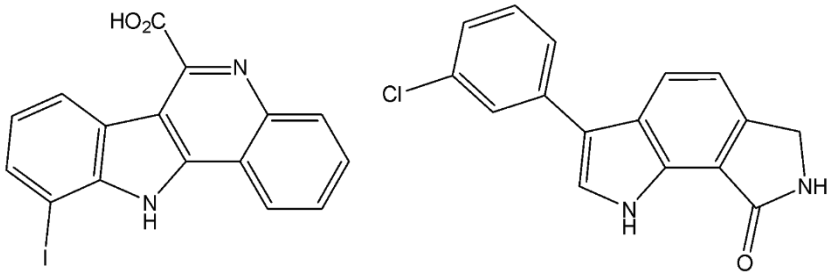


DYRK and CLK inhibitors: Is selectivity feasible?

Conrad KUNICK[1,2]

[1]Institut für Medizinische und Pharmazeutische Chemie, Technische Universität Braunschweig, Beethovenstraße 55, 38106 Braunschweig, Germany. [2]Center of Pharmaceutical Engeneering (PVZ), Technische Universität Braunschweig, Franz-Liszt-Straße 35A, 38106 Braunschweig, Germany.

Protein kinases belonging to the DYRK and the CLK families share high structural similarities of their ATP binding pockets. Thus, chemical inhibitors of DYRK and CLK kinases are typically not very selective for a distinct DYRK or CLK family member. While this could be a favorable property of a drug against neurodegenerative diseases in which hyperactivities of both CLK and DYRK kinases are involved, high selectivity of an agent is desirable when it is used as a chemical probe or as a tool in biological assays. In the course of our search for novel kinase inhibitory chemotypes we have identified the following two molecules with orthogonal DYRK and CLK inhibition selectivity. KuFal194 (**1**) is a highly selective DYRK1A inhibitor with single-digit nanomolar potency on the isolated enzyme. This molecule also exhibited activity in cellular assays, albeit in considerable higher (micromolar) concentrations (1). KuWal151 (**2**) was discovered as member of a novel compound class designated “mini-indirubines”. In contrast to the indirubin model compounds which are established GSK-3 inhibitors, most mini-indirubines inhibit non-selectively CLK and DYRK kinases. Among the mini-indirubines, **2** was selective for CLK1/4 without interfering with DYRK kinases (2). In the presentation, syntheses of the new inhibitors as well as models of their orientation in the ATP binding pockets of host kinases will be reported.



KuFal194 KuWal151

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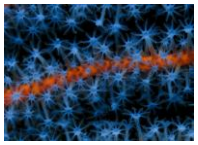

DYRK1A overexpression contributes to enhanced astrogliogenesis in a Down syndrome mouse model

Nobuhiro KURABAYASHI and Kamon SANADA

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Down syndrome (DS) is caused by trisomy for human chromosome 21. Individuals with DS commonly exhibit mental retardation, which is associated with anomalies in brain development. In the neocortex of DS brain, the density of neurons is remarkably reduced, whereas that of astrocytes is increased. Similar to abnormalities seen in DS brain, mouse models of DS show deficits in brain development, and neural progenitor cells that give rise to neurons and glia show mis-regulation in their differentiation. These suggest that the mis-regulation of progenitor differentiation contributes to alteration in numbers of neurons and astrocytes in DS brain. Nevertheless, the molecular basis underlying these defects remains largely unknown. We demonstrated that increased dosage of DYRK1A contributes to enhanced astrocytic differentiation of progenitors in the Ts1Cje mouse model of DS. Further, we link the increased dosage of DYRK1A to elevated activity of STAT, a transcription factor critical for astrogliogenesis. Together, our findings indicate that potentiation of the DYRK1A-STAT pathway in progenitors contributes to aberrant astrogliogenesis in DS.

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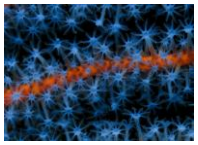
Funding: Grants-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and research grants from the Mitsubishi Foundation and Takeda Science Foundation



Identification of new DYRK2 substrates and their implications in carcinogenesis.

Maribel LARA-CHICA, Rosario MORRUGARES, Carla JIMENEZ-JIMENEZ, Eduardo MUNOS, Marco A. CALZADO CANALE

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Dual specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) is a relevant Ser/Thr kinase involved in the regulation of cell processes such as cytokinesis, cell proliferation and differentiation (1). Several studies have shown its important role in carcinogenesis, due to the ability to phosphorylate and regulate some relevant proteins implicated in this process, such as p53, c-Jun/c-Myc, SIAH2, Snail or Rpt3 (2, 3). These data have centred the attention of the scientific community on this kinase, being considered an “essential protein” in the control of tumorigenesis, and thus becoming a key candidate as a target for anticancer therapy. Nevertheless, there are few known substrates able to be regulated by DYRK2. In this sense, it is highly important to further study how this kinase exerts its function during tumour development and progression, through the identification of new substrates that take an active part in these processes.

To assess this point, we performed several technical approaches (kinase array and MS/MS) in order to identify new potential DYRK2 substrates. We obtained a list of relevant proteins involved in the different steps of carcinogenesis, including CDC25A among them. Given the essential role of this phosphatase as a key regulator of the cell-cycle progression, we focused on studying the effect of DYRK2 on CDC25A. Our study demonstrates that DYRK2 down-regulates CDC25A expression, facilitating its proteasomal degradation. This degradation depends on DYRK2 kinase activity, being this kinase the most effective in degrading CDC25A among the rest of the members of its family. This effect is independent of HIPK2 and ATM/ATR activities. An important inverse correlation of the expression of DYRK2/CDC25A was detected under different stimuli of the kinase and its specific silencing by siRNA. Furthermore, this inverse correlation was confirmed in lung cancer cells, which occurs specially in a cellular model system of bronchial epithelial cell squamous differentiation.

Taken together, our findings show DYRK2 as a new regulator of relevant proteins involved in tumour development and progression, such as CDC25A, what helps to improve our knowledge of the tumorigenic process and could open a road to the development of new therapeutic strategies against cancer.

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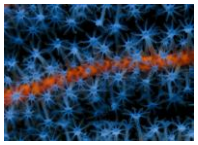
Financial support: this work was supported by MINECO (SAF2016-75228-R). MLC was supported by an FPU fellowship (FPU13/03393) from MECD.



Identification of a new function of Cystathionine β-Synthase (CBS) and characterization of its link with DYRK1A using *S. cerevisiae*

Alice LEON[1], Nadège LOAEC[1], Yann HERAULT[2,3], Marc BLONDEL[1], Gaëlle FRIOCOURT[1]

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Cystathionine β-Synthase (CBS) is an enzyme misregulated in two inherited intellectual deficiencies (ID): homocystinuria, which is caused by *CBS* loss-of-function mutations, and Down syndrome (DS), where *CBS* overexpression, due to its localization on the chromosome 21, would therefore be responsible for the cognitive phenotype of patients. In this latter disease, *CBS* along with *DYRK1A* are genes thought to contribute to ID in DS, and several studies in mouse suggest a genetic and probably functional link between these two genes.

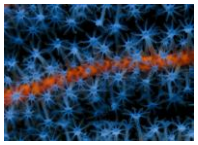
Modulating the activity of CBS is therefore a promising therapeutic strategy to reduce cognitive disorders of patients. Yet, to date efforts have failed to identify drugs that efficiently and specifically inhibit CBS activity *in vivo*, suggesting that CBS could be an “undruggable” target.

In order to identify cellular partners of CBS, as well as some of its potential modifier genes, we set up a genetic screen based on a yeast model overexpressing *CYS4,* the yeast homolog of *CBS*. Indeed, the identification of genes related to CBS, and particularly to the consequences of its overexpression, may help to define new therapeutic targets for CBS-related ID, in particular in DS patients.

Our genetic screens led to the identification of several genes that suggest a previously undescribed role for Cys4p/CBS in vesicular trafficking, which is a central pathway for synaptic transmission. In addition, we found that both *MCK1* and *YAK1*, the yeast homologs of human *GSK3* genes and *DYRK1A* respectively, modulate the phenotypic consequences of *CYS4* overexpression in yeast. These results suggest that the functional link between these genes, which has been highlighted in several studies, is conserved in *S. cerevisiae*.

A more extensive analysis of these pathways, first in yeast, then in mammalian cells, will help us to better understand how a misregulation of CBS activity can cause ID, and thus to propose new therapeutic strategies.

Financial support : AL is supported by the French « Ministère de l’Education Nationale, de l’Enseignement Supérieur et de la Recherche ». This work is funded by the Fondation Jérôme Lejeune and ITMO BCDE (Biologie Cellulaire, Développement et Evolution).



The role of DYRK1A in DNA repair

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The function of DYRK1A protein kinase is regulated by its gene dosage whereby both gains and losses of one copy of *DYRK1A* gene on chromosome 21 result in developmental abnormalities. In order to better understand the function and regulation of DYRK1A, we applied MudPIT proteomic approach to identify DYRK1A-interacting proteins in human cells. Four biological replicate experiments were performed to identify proteins reproducibly detected in the DYRK1A immunoprecipitates but not in the controls. Six proteins detected in all four biological replicate experiments were also most highly enriched in the DYRK1A immunoprecipitates, suggesting that these proteins form stable and abundant complexes with DYRK1A. One of these proteins, RNF169, has been recently characterized as a component of ubiquitin-mediated cascade involved in the repair of DNA double-strand breaks (DSBs). Presence of specific chromatin marks including ubiquitination regulates the choice between two major DSB repair pathways: homologous recombination repair (HRR) and non-homologous end joining (NHEJ), mediated by recruitment of chromatin-binding DNA damage response proteins including 53BP1 and RNF169. Binding of 53BP1 could prevent the resection of the DNA strands near the damage site necessary for the HRR while RNF169 is thought to promote the HRR by limiting 53BP1 accumulation. To determine whether DYRK1A plays a role in these processes, we knocked out its expression in human and mouse cell lines using CRISPR-Cas9 approach. We found that both the number of the 53BP1 IRIFs and their persistence over time were significantly reduced in the cell lines that lacked DYRK1A. This effect was dependent on the presence of RNF169, suggesting that DYRK1A regulates the ability of RNF169 to limit 53BP1's accumulation at the DSBs. Next, we sought to determine the mechanism of this regulation and found that RNF169 is phosphorylated by DYRK1A at two sites located in a highly conserved domain with no known function. Interestingly, the phospho mimetic mutant of RNF169 displayed a decreased ability to inhibit 53BP1 IRIF formation when compared to the wild type or the non-phosphorylatable RNF169 alleles. Since loss of DYRK1A could be relevant to cancer due to its widespread gene copy number losses, we determined the effect of DYRK1A loss on the ability of the cells to repair their DNA. Using the DR-GFP reporter of HRR and the neutral comet assays, we found that loss of DYRK1A results in an increased efficiency of the DNA DSB repair. Our findings implicate DYRK1A in the critical processes of DNA damage response and reveal a novel function of this important protein kinase.

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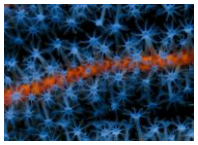

Multilayered proteomic analysis of cancer mutations in the Dyrk2 kinase complex

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Dual-specificity tyrosine-phosphorylation-regulated kinase 2 (Dyrk2) belongs to the class II (Dyrk2, Dyrk3, Dyrk4) kinases of the Dyrk family and is suggested to be involved in regulating key cellular processes such as cell proliferation, cytokinesis and cellular differentiation. Furthermore, Dyrk2 functions in the DNA damage response by phosphorylating p53 thereby promoting cellular apoptosis upon genotoxic stress (1). A dysregulation and mutations of Dyrk2 were found in various cancer types classifying Dyrk2 as potential oncogene. In addition to its kinase function Dyrk2 acts as a scaffold for the assembly of a multifunctional protein complex encompassing the ubiquitin ligase Ubr5 and the substrate receptor subunit DDB1-VprBP (2). The Dyrk2-dependent phosphorylation of recruited substrates is required for the subsequent ubiquitylation by the E3 ligase and its proteasomal degradation. Substrates of the Dyrk2 kinase complex are the AAA-ATPase katanin p60 acting in the reorganization of spindle microtubules during mitosis and the catalytic subunit of the telomerase (TERT) whose dysregulation is reported in HIV disease and cancer (3).

We performed a proteomic interaction analysis of the Dyrk2 kinase network using affinity purification mass spectrometry (AP-MS) and proximity-dependent biotin identification (BioID-MS) and identified about 80 high confident interactors, in particular factors of cell cycle regulation, apoptosis and nuclear transport. The integration of cancer associated point mutations into Dyrk2 affected the interaction network and caused a disassembly of the Dyrk2 kinase complex. Proteomic and phosphoproteomic profiling of wild-type and CRISPR/Cas9 engineered Dyrk2 knockout cells by label-free quantitative mass spectrometry (SWATH-MS) revealed 189 differentially regulated proteins and about 60 altered phosphopeptides. Next steps will include a phosphoproteomic “footprint” analysis of the various cancer related Dyrk2 mutants followed by a topological and structural analysis of the cancer perturbed kinase complex by cross-linking coupled mass spectrometry.

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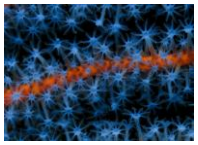

Leucettines, a family of DYRK1A inhibitors: from marine sponge to drug candidate

Laurent MEIJER[1], Thu Lan NGUYEN[1,2], Morgane CAM[1], Tania TAHTOUH[1], Emilie DURIEU[1], Emmanuelle LIMANTON[3], Benoît VILLIERS[1], Céline BRUYERE[1], Xavier FANT[4], Stefan KNAPP[5], Benoît SOUCHET[6], Jérôme BRAUDEAU[6,7], Antigoni MANOUSOPOULOU[8], Spiros D. GARBIS[8], Arnaud DUCHON[2], Yann HERAULT[2,9], François CARREAUX[3], Jean-Pierre BAZUREAU[3]

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There is growing evidence implicating DYRK1A in the onset and development of neurodegenerative pathologies such as Alzheimer's disease (AD) and Down syndrome (DS). Leucettines, an archetype of DYRKs inhibitors, will be reviewed in this presentation (1-3).

A random screen of natural products allowed us to identify the marine sponge Leucettamine B as an inhibitor of DYRKs. Synthesis of over 500 analogues (collectively referred to as Leucettines) led to a first optimized product, Leucettine L41. Leucettines were co-crystallized with DYRK1A, DYRK2, CLK3, PIM1 and GSK-3β. The selectivity of Leucettine L41 was extensively studied using 4 different methods. A SAR study was carried out with 78 Leucettines which were tested for their inhibitory action on 11 recombinant kinases and in five cellular assays (modulation of CLK1 pre-mRNA splicing, protection towards glutamate -induced cell death, induction of autophagy, phosphorylation of Tau Thr212, phosphorylation of cyclin D1 Thr286). Optimization of Leucettines towards a clinical drug candidate implies the development of an orally available drug able to cross the blood brain barrier while maintaining selectivity, potency and patentability. Recent progress in this direction will be presented.

Leucettine L41 was tested in two DS mouse models and 2 AD mouse models as will be presented by our collaborators. Both tgBACDyrk1a mice (a model expressing three DYRK1A gene copies) and Ts65Dn mice (a partial trisomy model) models overexpress DYRK1A and display cognitive impairment related to DS and AD. Leucettine L41 treatment led to normalization of the DYRK1A activity and fully corrected the deficits seen in these DS models. Leucettine L41 also prevented cognitive deficits triggered by icv injection of amyloid peptide Aβ25-35 as well as those observed in the APP/PS1ΔE9 transgenic mice model of AD. Extensive proteomic and phosphoproteomic studies are carried out to elucidate the molecular actions of Leucettine L41 in the brains of the mouse models as well as in cellular models (cells expressing human DYRK1A or kinase-dead DYRK1A). Altogether these results confirm that Leucettines are able to cross the blood brain barrier, to normalize DYRK1A activity, to modify specific phosphorylation patterns and to restore normal cognitive functions in 2 DS and 2 AD animal models.

Leucettines deserve further development as potential therapeutics against neurodegenerative diseases, and possibly other diseases,

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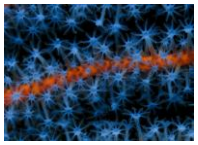

2-Substituted indole-3-carbonitriles as new DYRK inhibitors

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In our group the DYRK1A inhibitor 10-iodo-11*H*-indolo[3,2-*c*]quinoline-6-carboxylic acid was developed. It exhibits good inhibitory activity against DYRK1A (IC_{50} = 6 nM) and is highly selective against other DYRKs and CLK kinases. However, in cellular assays the DYRK1A inhibition was considerably lower because of poor physicochemical properties (1).

Here we report a fragment-based drug design starting from 7-chloro-1*H*-indole-3-carbonitrile as a small analogue of the 10-halogen substituted 11*H*-indolo[3,2-*c*]quinolone-6-carboxylic acids. During the development also water solubility and other physicochemical properties were taken into account. Analogous to the 11*H*-indolo[3,2-*c*]quinolone-6-carboxylic acids a 7-iodo substituent was favorable. Furthermore, various residues at the 1 and 2 position of the indole scaffold were introduced. A phenyl substituent in position 2 led to potent DYRK1A inhibitors. An additional methyl substitution at the indole nitrogen led to a very potent and selective DYRK1B inhibitor. The synthesis of the compound series, docking experiments and the results of enzyme assays will be presented.

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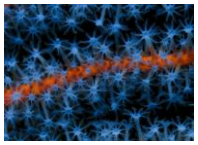
Financial support: this work was supported by the state of Lower Saxony, Germany, by a Georg-Christoph-Lichtenberg-Stipend in the graduate program “Processing of Poorly Soluble Drugs at Small Scale”.



Functional regulation of different DYRK family protein kinases by distinctive cellular binding partners

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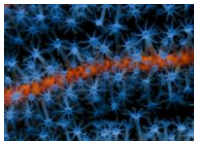
The human DYRK family consists of mutually-related five protein kinases, DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4. DYRK1A is encoded in the Down's syndrome critical region on human chromosome 21, and plays an important role in the functional and developmental regulation of many types of cells, including neuronal cells. Physiological roles of other members of DYRK family remain less evident. Here we report identification of cellular proteins that associate with specific members of DYRKs. We identified WDR68 (DCAF7), an evolutionarily conserved WD40-repeat protein, as a cellular binding partner of DYRK1A. WDR68 was originally identified in petunia as a factor controlling the transcription of flower anthocyanin biosynthetic genes. WDR68 was indispensable for the proliferation and survival of mammalian cells. DYRK1A and DYRK1B, but not other DYRKs, bound to WDR68 via the N-terminal domains. Importantly, DYRK1A-binding induced nuclear accumulation of WDR68. We then identified the molecular chaperone TRiC/CCT as a major WDR68-binding protein. Knockdown of cellular TRiC/CCT by siRNA caused an abnormal WDR68 structure and led to reduction of its DYRK1A-binding activity. Concomitantly, nuclear accumulation of WDR68 was suppressed by the knockdown of TRiC/CCT, and WDR68 formed cellular aggregates when overexpressed in the TRiC/CCT-deficient cells. Altogether, our results demonstrate that the molecular chaperone TRiC/CCT is essential for correct protein folding, DYRK1A-binding, and nuclear accumulation of WDR68.

We also found that molecular chaperones Hsp90, Cdc37, and Hsp70 associated with DYRK1B and DYRK4, but not with other DYRKs. Treatment of cells with an Hsp90 inhibitor geldanamycin induced dissociation of Hsp90 and Cdc37, but not Hsp70, from DYRK1B and DYRK4. DYRK1B and DYRK4 underwent formation of cytoplasmic aggregation and degradation by the Hsp90 inhibitor, suggesting that the chaperone function of Hsp90/Cdc37 is required for solubility and stability of these kinases. Altogether, we suggest that DYRK family protein kinases are distinctively regulated by respective binding partners in cells.



Developing inhibitors of the kinases that regulate alternative splicing

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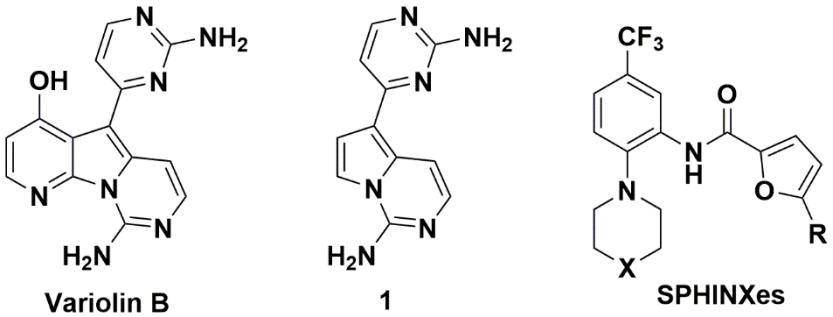
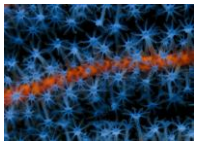
The splicing of pre-mRNA is one of the main processes that influence protein diversity in humans and recent studies have estimated that up to 88% of multi-exon protein coding genes are alternatively spliced. This diversity highlights the importance that alternative splicing plays in regulatory affairs, such as cell growth, differentiation and apoptosis. Alternative splicing is carried out by cellular machinery known as the spliceosome and this machinery is regulated by the phosphorylation of key splicing factors. By controlling the phosphorylation events, it is possible to regulate the production of protein isoforms.

To achieve this, we have been developing small molecule inhibitors of the kinases (such as the DYRKs, CLKs and SRPK1) responsible for the phosphorylation events. The pyrrolopyrimidine scaffold, as seen in variolin B and **1**, has been found to yield potent inhibitors of the CLKs and DYRKs. We have also identified other scaffolds that are potent SRPK1 inhibitors.



Preliminary biological data will be presented that demonstrates how some of these inhibitors can be used in the regulation of Treg differentiation and function, as well as in the regulation of the alternative splicing of VEGFA, one of the critical proteins involved in angiogenesis.



Deciphering the molecular mechanisms of action of pharmacological DYRK1A kinase inhibitors for therapeutic use in Down Syndrome preclinical models.

Thu Lan NGUYEN[1,2], Arnaud DUCHON[1], Antigoni MANOUSOPOULOU[4], Spiros D. GARBIS[4], Emmanuelle LIMANTON[5], François CARREAUX[5], Jean-Pierre BAZUREAU[5], Laurent MEIJER[2], Yann HERAULT[1,3]

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Down Syndrome or Trisomy 21 (DS), is due to the presence of an extra copy of chromosome 21. As the most frequent mental retardation it affects about 1 new born per 700 births.

Among the candidates implicated in DS intellectual disabilities, the Dual Specificity Tyrosine Phosphorylation Regulated Kinase, DYRK1A, found in the DS critical region of chromosome 21, is one of the most relevant (1). Indeed, several studies have shown a correlation between an increase of its kinase activity and the intellectual defects observed in DS models. EGCG, known to inhibit DYRK1A, was successfully used to rescue cognitive traits in mice and human (2, 3).

In order to understand the mechanisms underlying the impact of DYRK1A dosage on cognitive alterations, we used several trisomic mouse models expressing DYRK1A alone or with additional Hsa21 homologous genes and specific DYRK1A inhibitors from ManRos Therapeutics.

We will present here the consequence of treatments with Leucettine 41, a synthetic DYRK1A inhibitor, following repetitive administration to several DS mouse models on the behaviour and cognition and on several activities of DYRK1A. Further analysis of the phosphoproteome of DS mouse models treated or not with L41 unravels a few targets and pathways which are involved in the restoration of cognitive capacities of DS models, highlighting molecular synaptic mechanisms. These results support the potential of more selective DYRK1A inhibitor as a therapeutic approach to improve cognitive functions in DS patients.

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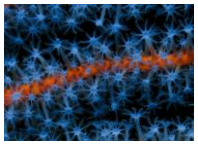
Financial support : CIFRE, FUI TRIAD, Fondation Jérôme Lejeune.



Multimodal regulation of the microtubule cytoskeleton by DYRK1a

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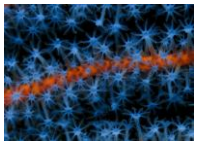
Cellular architecture is governed by the organization of cytoskeletal networks and determines the functional output of a cell. It is therefore essential to understand the regulatory mechanisms of cytoskeleton organization as a cell develops, changes, or maintains its internal structure, because altering these processes can disrupt cell function and ultimately lead to pathological conditions. We and others have shown that DYRK1a regulates the microtubule cytoskeleton directly by phosphorylating beta-tubulin, and indirectly through phosphorylation of the microtubule associated protein, Tau. We have utilized a chemical genetic approach to identify direct downstream substrates of *Drosophila* MNB in larval lysates, as well as mammalian DYRK1a in brain lysates, and discovered additional conserved proteins that act upon the microtubule cytoskeleton. Using in vitro kinase assays, single molecule TIRF microscopy, and in vivo neuronal imaging, we have begun to characterize the role of DYRK1a in shaping the microtubule landscape through differential regulation of the microtubule associated proteins, Tau and MAP7. By analyzing neurons from mitosis to neuronal differentiation and pruning, we hope to provide a complete map of the DYRK1a kinase network during neuronal development, and elucidate the multiple ways in which this kinase modulates the microtubule cytoskeleton during different cellular processes.

Financial support: this project is supported by NIH grant R00HD080981 to KMOM.



Drug-target engagement and efficacy of DYRK1A inhibitors in glioblastoma cells

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Glioblastoma is an aggressive brain tumour resistant to conventional chemo- and radiotherapy. All patients succumb to the disease within two years, and glioblastoma remains a major unmet medical need. Amplification and mutations of the epidermal growth factor receptor (EGFR) occur in over 60% of glioblastoma cases. Recent evidence suggests that to effectively target EGFR, complete degradation and removal of the receptor is necessary, as sole inhibition of the kinase catalytic function is insufficient to achieve anti-cancer effects (1, 2). Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) is up-regulated in glioblastomas and has been shown to prevent endocytotic degradation of EGFR (3). DYRK1A activity results in enhanced EGFR signalling and tumour growth. Therefore, inhibition of DYRK1A is a potential therapeutic intervention for EGFR-dependent glioblastomas. A series of novel DYRK1A inhibitors was designed and synthesized. The library was screened for their anti-cancer efficacy in established and patient-derived glioblastoma cell lines with varying EGFR expression. The most potent DYRK1A inhibitors in the series (IC_{50} ≤ 30 nM) decreased viability of numerous cell lines (EC_{50} ≤ 1 μM) and promoted EGFR degradation by decreasing its half-life by 3-fold. In addition to their anti-proliferative properties, the most potent inhibitors also reduced migration and invasion of glioblastoma cells. Target engagement was confirmed with genetic knockdown and the cellular thermal shift assay (CETSA) (4). We demonstrate that DYRK1A knockdown phenocopies activity of DYRK1A inhibitors in the EGFR degradation assay and DYRK1A's thermal stability in cells is increased upon drug treatment, confirming that these drugs bind to DYRK1A in cells. In summary, we present detailed pharmacology investigation of novel DYRK1A inhibitors and identification of lead compounds with anti-cancer properties necessary to combat glioblastoma.

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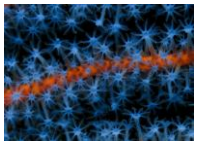

DYRK1A haploinsuficiency is a frequent cause of intellectual disability: How to better diagnose it?

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Heterozygous de novo loss-of-function mutations in *DYRK1A* cause a syndromic form of Intellectual disability (ID) associated with growth retardation including microcephaly, specific dysmorphy, stereotypy, ataxia and other variable clinical features (Mental Retardation, autosomal dominant 7, MRD7). MRD7 is a frequent cause of ID as loss-of-function mutations are found in around 0.5% of ID patients. Since recently, several *de novo* missense variations have been identified, sometimes in patients not highly evocative of MRD7 which raises the question of their true pathogenicity. Therefore, functional tests need to be developed to establish if these missense variants participate, fully or partially, to the cognitive impairments observed in these patients. *DYRK1A* encodes a protein serine/threonine kinase expressed throughout life, involved in many cellular processes, and particularly in the regulation of gene expression. We are performing transcriptomic studies to identify alterations of gene expression in patient's cells. This will allow to develop a functional assay useful to test the effect on DYRK1A function of the different missense variants identified. In parallel, we have undertaken to better define the clinical manifestations associated to DYRK1A mutations in French patients. This better delineation of the clinical spectrum of MRD7 and the development of functional tests will be useful to improve diagnosis of patients affected by this frequent form of ID. This will also allow to better understand the normal and pathological functions of DYRK1A.

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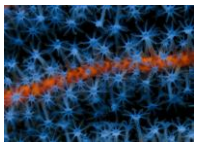

Interaction of the protein kinase DYRK1A with RNF169 suggests a role for this kinase in DNA repair

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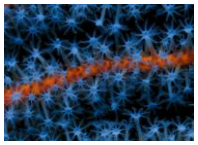
DYRK1A (dual-specificity tyrosine-regulated kinase) is overexpressed in Down syndrome individuals. This overexpression is proposed to be associated to risk of childhood leukemia and neurodevelopmental abnormalities. On the other hand, reduced levels of DYRK1A due to truncating mutations cause a rare human syndrome characterized by intrauterine growth retardation and microcephaly, among other clinical traits. Although the list of functional activities associated to DYRK1A has increased in recent years, a complete picture on DYRK1A physiological roles is still missing. To better understand DYRK1A activities an interactome analysis based on affinity DYRK1A purification coupled to mass spectrometry analysis was undertaken. RNF169 (*R*eally interesting *n*ew gene *f*inger protein 169), an E3-ubiquitin ligase described as a key component of the cellular response to double-strand breaks (DSBs), appeared as one of the top DYRK1A nuclear targets, and the functional characterization of this interaction has uncovered a novel physiological role for DYRK1A. DYRK1A interacts directly with RNF169 through a dedicated motif in the non-catalytic N-terminus, and this interaction is essential for the recruitment of DYRK1A to DSB sites. RNF169 is a substrate of DYRK1A *in vitro* and *in vivo*. Using a combination of mass spectrometry analysis, mutagenesis and *in vitro* kinase assays, several DYRK1A-dependent phosphosites have been identified in RNF169. Interestingly, DYRK1A knockdown leads to increased radiation sensitivity. Our results point to a possible role for DYRK1A in the regulation of DNA-damage response through phosphorylation of the E3 ubiquitin ligase RNF169.



Retinal phenotypes in murine models of Down syndrome

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The retina of Down syndrome patients is thicker than the one from controls, an anomaly reproduced in two murine models of the disease, Ts65Dn and Tg(*Dyrk1a*) and attributed to the increased dosage of *Dyrk1a* (1). As HSA21 genes not triplicated in Ts65Dn may affect the expression/function of DYRK1A, we have analyzed by optical coherence tomography (OCT) the retina of Ts65Dn and Tg(*Dyrk1a*) mice, and compared the results to those obtained on various additional models, notably Dp(16)1 Yeh mice, which have a triplicated segment of MMU16 longer than Ts65Dn, and the crosses between Tg(*Dyrk1a*) and Dp1Yah, which carries a triplication of the MMU17 genes orthologue to HSA21. As Down syndrome patients present anterior segment defects, as myopia and keratoconus, at a higher frequency than controls, we also measured by OCT corneal thickness, as well as anterior chamber and vitreous chamber depths. In parallel, we have started to analyze by immunohistochemistry the retinal neuron populations in Tg(*Dyrk1a*) mice, showing that most of the increase in retinal thickness is due to changes in the amacrine cell population, which is more affected than bipolar cells. Mirror results were obtained on the retina of *Dyrk1a*$^{+/-}$ mice, in which the amacrine cell population is strongly reduced, as the retinal thickness.

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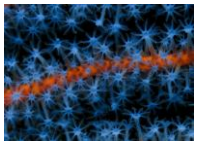

DYRK1B and Hedgehog signaling: a complex crosstalk

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Hedgehog (Hh) signaling plays important roles in embryonic development and in tumor formation. Apart from the well-established stimulation of the GLI family of transcription factors, Hh ligands promote the phosphorylation and activation of mTOR and AKT kinases, yet the molecular mechanism underlying these processes are unknown. Here, we identify the DYRK1B kinase as a mediator between Hh signaling and mTOR/AKT activation. In fibroblasts, Hh signaling induces DYRK1B protein expression, resulting in activation of the mTOR/AKT kinase signaling arm. Furthermore, DYRK1B exerts positive and negative feedback regulation on the Hh pathway itself: It negatively interferes with SMO-elicited canonical Hh signaling, while at the same time it provides positive feed-forward functions by promoting AKT-mediated GLI stability. Due to the fact that the mTOR/AKT pathway is itself subject to strong negative feedback regulation, pharmacological inhibition of DYRK1B results in initial upregulation followed by downregulation of AKT phosphorylation and GLI stabilization. Addressing this issue therapeutically, we show that a pharmacological approach combining a DYRK1B antagonist with an mTOR/AKT inhibitor results in strong GLI1 targeting and in pronounced cytotoxicity in human pancreatic and ovarian cancer cells.

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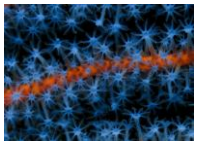

Leishmania infantum DYRK1: a negative regulator of the G1 to S cell-cycle transition, essential for the development of infective stationary phase promastigotes

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Leishmaniases are devastating infectious diseases caused by protozoan parasites of the genus *Leishmania*. *Leishmania* has a digenetic life cycle alternating between promastigote forms, which develop in the sand-fly vector, and an amastigote form, which grows in mammals after being bitten by an infected sand-fly. In the promastigote form cell cycle arrest in the G1 phase is an important process whereby *Leishmania* transforms from poorly infective into highly infective parasites. Metacyclics are metabolically and biochemically mimicked by stationary phase promastigotes. Dual-specificity tyrosine-regulated kinases *(*DYRKs*)* are known to act as negative regulators of growth and as positive regulators of cell differentiation (1). Interestingly, a *Leishmania* DYRK1 homologue (*Lin*J.15.0180) contains both DYRK specific and *Leishmania* specific- domains. In this study, we used transgenic *L.infantum* parasites that over-express *Lin*DYRK1. *Lin*DYRK1over-expression caused a significant delay in proliferation, associated with a delayed cell-cycle G1/S transition. To knockout *Lin*DYRK1, as loss-of-function analyses are often lethal, we used an established facilitated *Lin*DYRK1 knockout approach that relies on the episomal expression of our gene from an episome that is susceptible to drug induced negative selection. Persistence of the episome in logarithmic parasites during negative selection was used as a readout of essentiality. Loss of the *LinDYRK1* expressing episome in logarithmic parasites, was observed albeit at a low rate, enabling us to conclude that *Lin*DYRK1 deletion could be compensated. Phenotype analyses of *Lin*DYRK1 knockout parasites showed that parasites exhibited a marked thermosensitivity to a 26→37°C temperature shift, a natural environmental stress-factor occurring during stage differentiation. Moreover, *Lin*DYRK1$^{-/-}$ parasites displayed defects in stationary growth phase as they rounded up, showing an abnormal cell-cycle distribution with a marked increase of G2-M/G1 ratio, and severe surface aberrations, accumulation of vesicular structures and lipid body formation. Knockout mutants exhibited subtle but clear differences in the lipid composition that could account for lipid body emulsion size and thermosensitivity. Finally, null mutants showed a pronounced reduction in their ability to survive in mouse macrophages. Overall this work highlights the role of *Lin*DYRK1 in mediating the growth-stress response balance, to promote pro-survival and fitness of infective stationary phase parasites.

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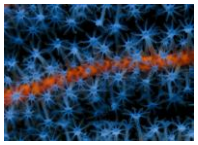

Preventing DYRK1A catabolism in reactive astrocytes as a novel therapeutic approach to treat Alzheimer's Disease.

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DYRK1A, dual specificity tyrosine phosphorylation regulated kinase 1 A, is a broad spectrum kinase involved in the phosphorylation of numerous proteins including TAU proteins, APP processing and neuroinflammation-related proteins. Altogether DYRK1A plays a crucial role in the pathogenesis of Alzheimer's disease (AD), the most common form of dementia. According to these protein targets, DYRK1A is a kinase suspected to be a potential target to treat AD. We thus, investigated DYRK1A in AD. In human hippocampal biopsies, we first identified an original DYRK1A catabolism leading to decrease DYRK1A full length protein levels in AD patients through Calpain II overactivation as previously observed (1). In addition, the resulting truncated DYRK1A forms accumulate in "astrocytes like" cells. In a widely used transgenic AD mice model (APPswe, PSEN1dE9; APP/PS1), we then confirmed this catabolism to be located in reactive astrocytes. Pharmacological targeting of DYRK1A using the Leucettine L41 compound, previously described *in vitro* as a DYRK1A inhibitor (2), prevented DYRK1A catabolism in reactive astrocytes. While astrogliosis was not modified, L41 treatment promoted the recruitment of phagocytosis-specialized microglia releasing low levels of inflammatory mediators. Of note, this tissue remodeling was associated with a decrease of amyloid-plaque load, synaptic plasticity improvement and cognitive abilities rescue. Surprisingly, no difference in the total endogenous DYRK1A activity was observed in AD patients or vehicle and L41 treated APP/PS1 mice relative to matched controls. Therefore, our study validates a AD therapeutic strategy targeting DYRK1A. However, we also provide the first evidence that it would be preferable to orient future investigations towards the DYRK1A catabolism in reactive astrocytes to treat this devastating disease.

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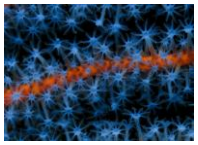

De novo mutations of DYRK1A lead to a syndromic form of ID

Bregje WM VAN BON

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Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (*DYRK1A*) maps to the Down syndrome critical region; copy number increase of this gene are thought to play a major role in the neurocognitive deficits associated with Trisomy 21. In 2011, we investigated whether smaller copy number variations were present at the *DYRK1A* locus in 3000 individuals and could confirm one *de novo* intragenic deletion of 52 kb in an individual who presented with intellectual disability (ID), microcephaly, a broad based gait and specific behavior (1). We noted a striking similarity with features previously observed in different animal models with mutated *Dyrk1A.*

Further studies of patients with ID and autism spectrum disorder (ASD) confirmed truncation of *DYRK1A* is associated with loss-of-function mutations. To understand the phenotypic spectrum associated with *DYRK1A* mutations, we resequenced the gene in 7,162 ASD/DD patients and 2,169 unaffected siblings (2). Comparison of our data and published cases with 8,696 controls identified a significant enrichment of *DYRK1A* truncating mutations ($p = 0.00851$) and an excess of *de novo* mutations ($p = 2.53\times10^{-10}$) among ASD/ID patients. Phenotypic comparison of all novel and literature cases identified a syndromal disorder including ID, microcephaly, ASD, stereotypic behavior and apparent feeding problems.

In general, the level of ID is variable (3). The majority of individuals function in the moderate to severe range of ID and a few individuals presented with mild ID. All individuals experienced apparent speech problems; notably, expressive language was more severely affected compared to receptive language. The majority of individuals show typical behavior including autism spectrum disorder, anxious and stereotypic behavior. Feeding and sleeping problems are common and may persist in adulthood.

In addition, intrauterine growth retardation, seizures and gait disturbances were frequently noted. About half of affected individuals developed epilepsy including atonic attacks, absences and generalized myoclonic seizures. Motor development is often impaired by gait disturbances and hypertonia. Although some individuals achieve independent walking at the upper age limit of normal, the majority achieve walking after age two to three years.

Finally, in almost all individuals a specific facial gestalt could be recognized, especially at an older age. During infancy and childhood, the face is characterized by deep-set eyes, mild upslanting palpebral fissures, a short nose with a broad tip, and retrognathia with a broad chin. In adulthood, the nasal bridge becomes high and the alae nasi short, giving the nose a more prominent appearance.

During my presentation I will focus on the clinical findings associated with DYRK1A loss of function mutations and discuss possible pitfalls in molecular diagnosis.

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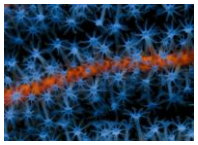

Inhibition of DYRK1A in the pancreatic beta cell

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Restoring functional beta-cell mass is an important therapeutic goal for both type 1 and type 2 diabetes. While proliferation of existing beta cells is the primary means of beta-cell replacement in rodents, it has been unclear whether a similar principle applies to humans, as human beta cells are remarkably resistant to stimulation of division. In order to identify small molecules capable of inducing beta-cell proliferation, we developed a human islet cell culture system suitable for high-throughput screening. Using this system, we found that 5-iodotubercidin (5-IT), an annotated adenosine kinase inhibitor, strongly and selectively increases human beta-cell proliferation *in vitro* and *in vivo*. Remarkably, 5-IT also increased glucose-dependent insulin secretion after prolonged treatment. Kinome profiling revealed 5-IT to be a potent and selective inhibitor of the dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) and cell division cycle (CDC)-like (CLK) kinase families. Induction of beta-cell proliferation by either 5-IT or harmine, another natural-product DYRK1A inhibitor, was suppressed by co-incubation with the calcineurin inhibitor FK506, suggesting involvement of DYRK1A and NFAT signaling. Gene-expression profiling in whole islets treated with 5-IT revealed induction of proliferation- and cell cycle-related genes, suggesting that true proliferation is induced by 5-IT. Furthermore, 5-IT promotes beta-cell proliferation in human islets grafted under the kidney capsule of NOD-*scid* IL2Rg^{null} mice. These effects are selective to the beta cell, pointing to DYRK1A inhibition as a therapeutic strategy to increase human beta-cell proliferation.

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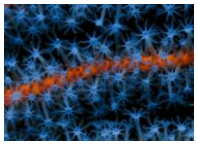

Tumor suppressive function of DYRK2

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Escape from apoptotic induction, dysregulation of cell cycle machinery, and acquisition of cell migration ability are major features on cancer development. These characteristics are brought by dysregulation of cellular homeostasis. Since the intracellular signal pathways crosstalk each other to maintain the homeostasis, molecular-based studies are required to better understand the mechanism for tumorigenesis. Dual specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) is a Ser/Thr kinase, and the intracellular functions had not been elucidated for decades; however, recent studies have shown that DYRK2 physiologically engages in these signal transductions. I will discuss the tumor suppressive functions of DYRK2 in several aspects such as apoptosis induction, cell cycle regulation, metastasis, and cancer stemness.

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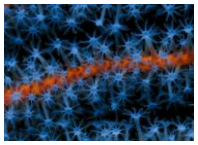

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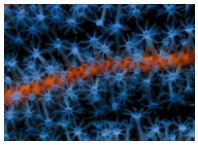

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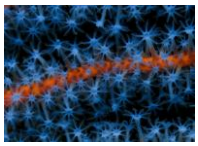

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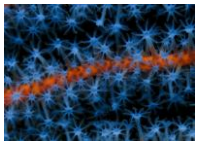

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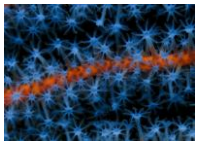

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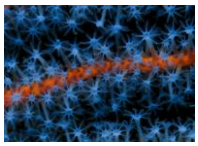

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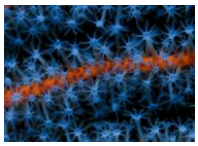

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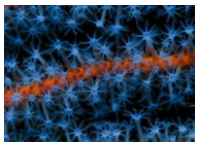

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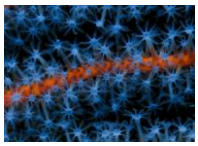

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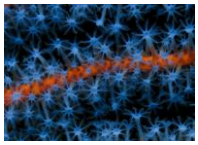

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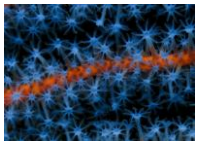

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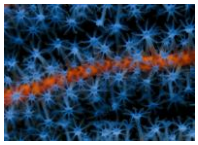

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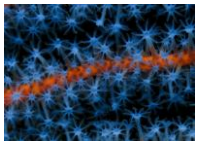

Cover : Whip Coral (Ellisella sp.) - showing polyp detail. Found throughout the Indo-Pacific. Photo taken off Anilao, Philippines. Within the Coral Triangle.

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