Quantifying dopamine D2 and adenosine A2A receptor interactions in rat brain using MolBoolean™ technology

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#8217

NEUROSCIENCE 2024

Introduction

Investigating protein-protein interactions (PPIs) is crucial for understanding normal neuronal functions and their dysregulation in neurological disorders.

In the striatum, adenosine via the A2A receptors antagonizes dopamine neurotransmission mediated by the DRD2 receptors. The receptors have been shown to form heterodimer complexes, which are functionally different from the individual receptors and have been suggested as a target for Parkinson's disease treatment.

In the present study, we utilized MolBoolean™, a newly-developed *in situ* proximity assay, to simultaneously detect free and interacting protein fractions of the dopamine D2 (DRD2) and adenosine A2A (ADORA2A) receptors in rat brain.

Materials and Methods

Tissues

FFPE sections from human TMAs containing 21 normal tissues and rat brains (n=3)

Antibodies

Anti-ADORA2A (Ab 05-717, mouse monoclonal, Thermo Fisher Scientific), Anti-DRD2 (HPA015691, rabbit polyclonal, Atlas Antibodies), Anti-GAD1 (AMAb91079, mouse monoclonal, Atlas Antibodies) and Anti-GFAP (AMAb91033, mouse monoclonal, Atlas Antibodies)

Antibody validation

Antibody specificity and selectivity was validated in human and rat tissues using chromogenic IHC

Protein-protein interactions

MolBoolean™ technology was used to assess the PPIs between DRD2 and ADORA2A receptors in the rat brain (n=3)

Cell type delineation

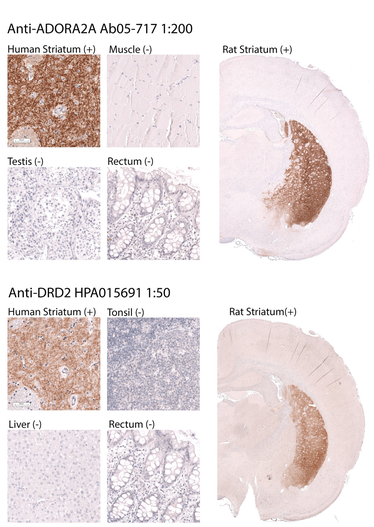
Indirect immunofluorescence (IHC-IF) was used to label neurons (GAD1) and astrocytes (GFAP)

Image acquisition and analysis

Image acquisition was performed using Leica laser scanning microscopy (4 images/rat in the striatum and 5 images/rat in the cerebral cortex)
Image deconvolution was performed using ImageJ
Results were quantified using a MolBoolean pipeline in the Cell Profiler image analysis software

Antibody Validation

ADORA2A and DRD2 antibody validation in human and rat tissues using immunohistochemistry



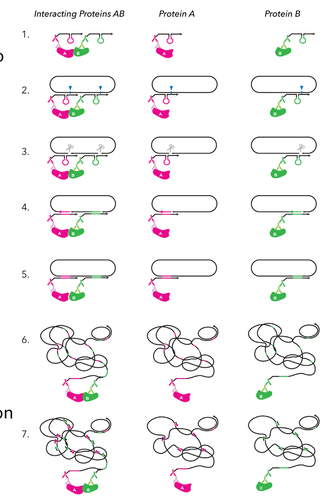
Chromogenic IHC images showing expected positivity in human and rat striatum (positive control) and absence of immunoreactivity in negative control tissues.

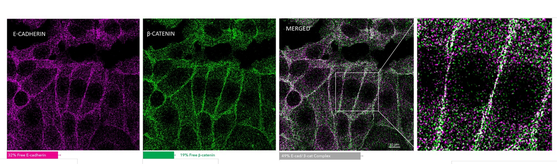
MolBoolean Technology

Simultaneous detection of free and interacting fractions of two protein targets

Assay steps

1. Proximity probe binding to primary antibodies
2. Proximity probe arm hybridization to DNA circle oligo
3. DNA nicking
4. Tag oligo incorporation
5. DNA ligation
6. Rolling Circle Amplification
7. Detection

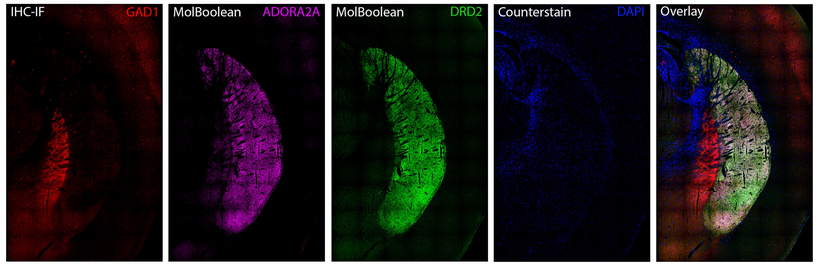




Example of E-cadherin and beta-catenin PPI analysis using MolBoolean™ technology. Magenta and green colors indicate individual proteins, while white color indicates PPI complex. Relative amounts of free and interacting receptors are indicated in the bar chart below.

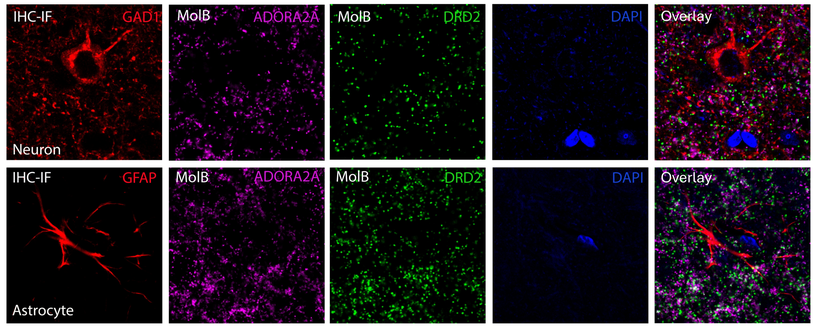
Results

ADORA2A and DRD2 receptor interaction in the rat striatum



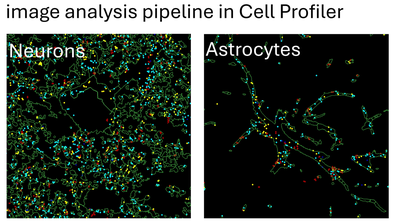
Low-power magnification image showing immunofluorescence detection of ADORA2A and DRD2 receptor interactions using the MolBoolean assay in the rat brain. The GABAergic neurons and processes are visualized by indirect IHC-IF using Anti-GAD1 antibody AMAb91079 (in red). ADORA2A and DRD2 expression is visualized using Anti-ADORA2A antibody Ab 05-717 (in magenta) and HPA015691 (in green) respectively, followed by MolBoolean detection. Nuclei were counterstained by DAPI (in blue). Interacting DRD2 and ADORA2A receptors are visualized as white signal on the overlay image.

ADORA2A and DRD2 receptor interaction in the striatal GABAergic neurons and astrocytes



High-power magnification confocal images showing immunofluorescence detection of ADORA2A and DRD2 interactions in rat striatal neurons (upper panel) and astrocytes (lower panel). The GABAergic neurons and processes are visualized by indirect IHC-IF using Anti-GAD1 antibody AMAb91079 (in red, upper panel)). Astrocytes are visualized using by indirect IHC-IF using Anti-GFAP antibody AMAb91033 (in red, lower panel)). ADORA2A and DRD2 expression is visualized using Anti-ADORA2A antibody Ab 05-717 (in magenta) and Anti-DRD2 antibody HPA015691 (in green) respectively, followed by MolBoolean detection. Nuclei were counterstained by DAPI (in blue). Interacting DRD2 and ADORA2A receptors are visualized as white signal on the overlay images.

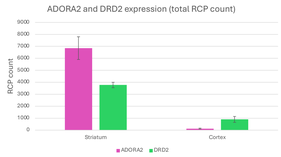
Automated quantification of free and interacting ADORA2A and DRD2 using the MolBoolean image analysis pipeline in Cell Profiler



Images showing automated quantification using Cell Profiler image analysis. Cell segmentation based on GABAegic neuronal marker (GAD1, left panel) or astrocyte marker (GFAP, right panel) is visualized in green.

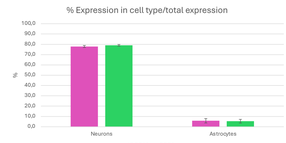
Light blue objects represent free ADORA2A,
Yellow objects indicate free DRD2,
Red objects display ADORA2/DRD2 complexes,
Dark blue objects show background signal.

Total ADORA2A and DRD2 expression in striatum and cortex



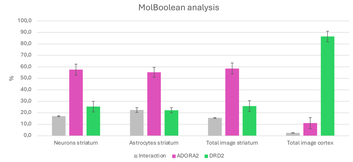
MolBoolean analysis demonstrates relatively high expression of DRD2 and ADORA2A receptors in the striatum, while cortex displays low expression of the receptors.

ADORA2A and DRD2 expression in neurons and astrocytes subpopulations in the striatum



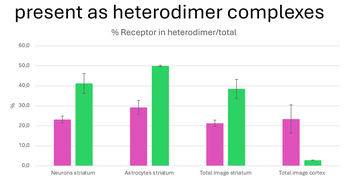
MolBoolean analysis demonstrates a high percentage of total ADORA2A and DRD2 receptor expression in neurons, while astrocytes displayed relatively low percentage.

Relative ADORA2A and DRD2 receptor interaction levels



MolBoolean data indicates that approximately 20% of DRD2 and ADORA2A receptors are interacting in both neurons and astrocytes in the striatum. 55% of ADORA2A and 25% of DRD2 are present as free proteins. In cerebral cortex, DRD2-ADORA2A interactions are markedly lower, about 2.5%.

ADORA2A and DRD2 receptors present as heterodimer complexes



MolBoolean analysis indicates that relatively high percentage of total DRD2 in complex with ADORA2A is evident in the striatum, while the reverse is observed in the cortex.

Conclusions

MolBoolean™ technology successfully detected both individual and interacting ADORA2A and DRD2 proteins in the rat brain. Furthermore, the combination of MolBoolean with IHC-IF enabled PPI quantification in the distinct cell subpopulations. MolBoolean™ technology is a powerful tool for dissecting PPIs underlying normal brain functions and their dysregulation in neurological disorders.

ATLAS ANTIBODIES

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