Analysis of human biopsy specimens in a hospital by HR-MAS NMR
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OUTLINE

• General principles of HR-MAS NMR

• Practical aspects of a metabolomic analysis by HR-MAS NMR

• Analysis of human biopsy specimens (Strasbourg Hospital):
  ✓ Sample collection and preparation
  ✓ Clinical research projects
    ➢ Pheochromocytomas
    ➢ Glioma brain tumors (*ExtempoNMR Project*)
General Principles of HR-MAS NMR
Assessing the metabolic content of unprocessed human biopsies by NMR?

• The NMR signals of metabolites in biopsies are naturally broad under static conditions
• The heterogeneity of the sample locally distorts the magnetic field lines
• Biopsy specimens cannot be studied with classical high-resolution NMR methods
• A specific NMR technique is required
Principles of High Resolution Magic Angle Spinning (HR-MAS) NMR

- Study of mobile small molecules contained within heterogeneous substances
- Main interaction: Distribution of magnetic susceptibilities ➔ Broad NMR lines
- Interaction removed by spinning the sample at the magic angle
  \( (3 \cos^2 \theta - 1) = 0 \) ➔ \( \theta = 54.7^\circ \)

Practical aspects of HR-MAS NMR
High Resolution Magic Angle Spinning (HR-MAS) NMR
Required equipment

The sample container

The probe

Pneumatic Unit

Sample changer
Main fields of application of Magic Angle Spinning (HR-MAS) NMR

- Biopsies
- Cells
- Swollen Polymers
- Food stuff
- Swollen Solid phase synthesis compounds
- Chromatographic beads
- ...
Data acquisition: Experimental conditions for Biopsy studies
1D $^1$H HRMAS spectrum of an Oligodendroglioma biopsy sample

Temperature: 276 K
Rotation: 3.5 kHz
Acquisition time: 20 min
Sample weight: 15 mg
⇒ 35 to 45 metabolites detected
Data acquisition: Experimental conditions

Critical parameters:

- Temperature: 276 K
- Rotation: 3.5 kHz
- Acquisition time for 1D CPMG spectrum: 20 min

Preserve sample integrity

- Metabolites composition
- Structure of the tissue (subsequent histological analysis)
Long duration 2D NMR experiments

⇒ HR-MAS NMR at low speeds of rotation

- 1H/13C HSQC experiment recorded for 18 hours at a speed
- $\omega_R = 500$ Hz
- No SSB
- Sample integrity preserved over 15h
- ⇒ Possible future general use in 2D NMR

Liver tissue sample

M. Renault et al., *Scientific Reports*, 2013, 3, 3349 & M. André et al., *Analytical Chemistry*, 2014 86 (21), 10749
Data analysis: Two approaches are combined

Quantitative Methods

Multivariate statistical analysis (PCA, PLS...)
Analysis of human biopsy specimens in Strasbourg Hospital by HR-MAS NMR

Collaborative project:
Strasbourg University: K. ElBayed
Spectrometer located in the histopathology department close to the operating theater

- Access to the tumor bank of the histopathology department

- Longitudinal study of patients possible

Cancer projects:
- Colons
- CNS tumors: Oligodendrogliomas,
  Glioblastomas
- Neuroblastomas (pediatric tumor)
- Kidneys
- Breasts
- ...
Protocol for biopsy specimens collection

1. Surgical operation
   → Histopathology department

2. Histopathological Analysis/Diagnosis
   → HRMAS analysis
      Storage -80 °C
      (Tumor bank)

3. Biomolecular analysis
   Storage -80 °C

4. Rest of the sample:
   Storage -80 °C
   (Tumor bank)

Clinical Research Projects in Strasbourg Hospital
1) Metabolic profiling of Pheochromocytomas/Paragangliomas: Detection of \textit{Succinate Dehydrogenase} (SDH) deficiency

Alessio Imperiale, MD Ph.D
Pheochromocytomas/Paragangliomas tumors: Medical aspects

- Pheochromocytomas/Paragangliomas (PHEOs/PGLs) are closely related rare neuroendocrine tumors

- Pheochromocytomas develop in the inner region (medulla) of the adrenal gland where hormones such as adrenaline and noradrenaline are secreted

- Of all the known genetic mutations, deleterious mutations in any of the succinate dehydrogenase (SDH) genes are currently the leading genetic cause of head and neck PGLs and account for more than 30% of hereditary cases

- Other PHEOs/PGLs are sporadic (not inherited)

SDHx and sporadic tumors are hard to differentiate

Patients with SDHx and sporadic tumors receive different treatments

A. Imperiale et al., Neoplasia (2015) 17, 55-65
Succinate Dehydrogenase (SDH) is an enzyme that catalyzes the oxidation of succinate to fumarate in the Krebs cycle.

\[ \text{Succinate} \xrightarrow{\text{Succinate Dehydrogenase}} \text{Fumarate (trans-isomer)} \]

\[ \Delta G^\circ = 0.0 \text{kJ/mol} \]
Key medical question:
- Can we differentiate the metabolic profile of SDH patients from sporadic PHEOs/PGLs?
- Can we identify specific metabolites?

Protocol of the study:
- 71 patients: 48 sporadic (42 PHEOs, 6 sympathetic PGLs), 23 SDHx
Representative 1D $^1$H HR-MAS NMR spectrum of a $SDHx$ tumor and of a sporadic tumor.

$SDHx$-tumor

Sporadic-tumor
Representative 1D $^1$H HR-MAS NMR spectrum of a $SDHx$ tumor and of a sporadic tumor

$SDHx$-tumor

$SDH$ catalyzes the oxidation of succinate to fumarate in the Krebs cycle

$\Rightarrow$ A $SDH$ mutation will lead to an accumulation of succinate in the tissue
Detection of SDH mutation by 1D $^1$H HR-MAS NMR

- SDH mutation can be readily detected by HR-MAS NMR on biopsy specimens
- The procedure can be easily implemented in a hospital to analyze biopsy specimens of Pheochromocytomas/Paragangliomas
- Obviously, detecting this mutation in vivo by MRI/MRS would also be of interest … Succinate is a good candidate for magnetic resonance spectroscopy (MRS)
Detection of SDH mutation by 1D $^1$H HR-MAS NMR

The natural link with \textit{in vivo} magnetic resonance spectroscopy

- SDH mutation can be readily detected by HR-MAS NMR on biopsy specimens
- The procedure can be easily implemented in a hospital to analyze biopsy specimens of Pheochromocytomas/Paragangliomas
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\textbf{Clinical Cancer Research (2016)}

\textit{“In Vivo Detection of Succinate by Magnetic Resonance Spectroscopy as a Hallmark of SDHx Mutations in Paraganglioma”} by Lussey-Lepoutre \textit{et al.}
2) HR-MAS NMR analysis of human Glioma biopsies

*ExtempoNMR* Project
Classification of Gliomas (human brain tumors)
Patient treatment / Survival prognosis

- Gliomas represent about 30% of brain tumors and 80% of malignant brain tumors
- Gliomas are classified according to the type of cell with which they share histological features and to their grade (WHO: II/III/IV)
- *The histopathological classification defines the patient treatment and the survival prognosis*

- Histopathological classification of Gliomas:
  - Oligodendroglioma II (Grade II)
    - long survival time
  - Oligodendroglioma III (Grade III)
    - intermediate survival time
  - Glioblastoma (Grade IV)
    - short survival time (about 14 months)

Key medical questions:

- Does the metabolic classification agree with the histopathological classification?
- Is the clinical evolution of the patient (*Overall Survival*) in agreement with the metabolic classification?

Cohort studied (under constant evolution):

- Oligodendroglioma II (Grade II) (n=40)
- Oligodendroglioma III (Grade III) (n=81)
- Glioblastoma (Grade IV) (n=183)
- Healthy Cortex (n=48)
Average 1D $^1$H HR-MAS NMR spectra of grade II/III/IV gliomas

A. Elkhaled et al., NMR in Biomedicine (2014) 27, 578-593
Analysis of 1D $^1$H HR-MAS NMR spectra of grade II/III/IV gliomas

Clinical evolution of the patient

Unsupervised hierarchical clustering of human brain gliomas: 
*Control/ODII /ODIII /Glioblastoma*

Similar spectral pattern for Cortex
Analysis of 1D $^1$H HR-MAS NMR spectra of grade II/III/IV gliomas

Clinical evolution of the patient

Unsupervised hierarchical clustering of human brain gliomas:
Control/ODII /ODIII /Glioblastoma
Metabolomics by HR-MAS NMR in neuro-oncology
Hierarchical clustering of human brain gliomas:
*Overall Survival using* metabolomic clustering (Kaplan-Meier plot)
Biomarker detection (targeted analysis):
IDH1 mutation in patients with grade II/III/IV gliomas
Indicator of good prognosis

- Isocitrate dehydrogenase (IDH1) mutation is present in secondary glioblastomas and grade II/III gliomas

- IDH1 mutation is a marker of good prognosis

- There is a significant difference in the probability of survival for patients with IDH mutation (GBM: 31 vs. 14 months, grade III glioma: 65 vs. 20 months)

  ➔ IDH mutation leads to an accumulation of 2-Hydroxyglutarate (2-HG) in gliomas

  ➔ 2-HG can be detected by $^1$H HR-MAS NMR

Detection of IDH mutation in Glioma patients by HR-MAS NMR
Indicator of good prognosis

IDH mutation leads to an accumulation of 2-Hydroxyglutarate (2-HG)

Detection of IDH mutation in Glioma patients by HR-MAS NMR
Indicator of good prognosis
Better Detection of 2-HG using a 2D J-resolved experiment
Overall survival function using the level of 2-HG
In Oligodendrogliomas of grade II/III of the cohort studied

P-value = 2e-06
Overall survival function using the level of 2-HG
In Oligodendrogliomas of grade II/III of the cohort studied

Strong implications in terms of patient management (neurosurgery)
Towards real-time HR-MAS NMR analysis of human brain biopsies during a neurosurgical operation

ExtempoNMR Project
Strasbourg University Hospitals

ExtempoNMR Project:
Real-time metabolic profiling of biopsy specimens during neurosurgery

• Provide in real-time relevant information to the neurosurgeon

• Rapid analysis of brain biopsy specimens by HR-MAS NMR (15 min)

• Possible information:
  ➢ Degree of aggressiveness of the tumor (prognosis/survival time)
  ➢ Detection of biomarkers (2-HG)
  ➢ Detection of cancer tissue to better delineate the tumor margins
ExtempoNMR Project:
Metabolomics by HRMAS NMR in neuro-oncology
Real-time analysis

Patient 2: exeresis GBM
**ExtempoNMR Project:**
Metabolomics by HRMAS NMR in neuro-oncology
Real-time analysis

Patient 2: exeresis GBM

**Histopathology (tumoral infiltration)**
- T2: 80-90%
- R1: close to normal tissue
- R2: 70%
- R3: <5%
- R4: <5%
Strasbourg University Hospitals:
Installation of a pneumatic tube carrier to rapidly transfer biopsy specimens from the operating theater to the NMR spectrometer

- Rapid analysis of brain biopsy specimens by HR-MAS NMR (15 to 20 min)
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