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► **To cite this version:**

Eric Rivals. Analysis of epitranscriptome for grading of glioma. Symposium on Cancer genomics and epitranscriptomics: from the bench to the clinic, Comprehensive Cancer Center (CIC), University of Salamanca, Nov 2022, Salamanca, Spain. <lirmm-04287393>

HAL Id: lirmm-04287393

<https://hal-lirmm.ccsd.cnrs.fr/lirmm-04287393v1>

Submitted on 15 Nov 2023

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Analysis of epitranscriptome for grading of glioma

Eric Rivals

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UNIVERSITÉ
DE MONTPELLIER

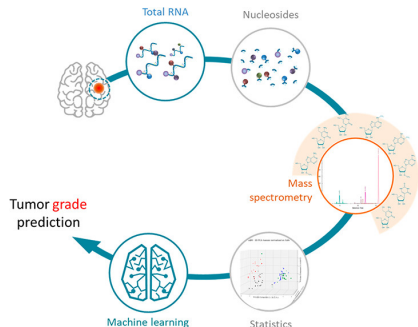


Objective and summary

Glioma: the most frequent brain cancer, is difficult to cure and to follow up.

Aim:

1. determine whether a **panel** of epitranscriptomic marks can serve as biomarkers for **glioma grading**
2. pipeline for grade prediction using epitranscriptomic profile



[7]

Diffuse glioma: the most frequent of brain tumors

Glioma: intracranial tumor of glial cells

- ▶ Diverse forms and locations in the brain.
- ▶ 2nd most frequent tumor among child cancers
- ▶ Diagnostic: using Magnetic Resonance Imaging (MRI), scanner, and biopsy.
- ▶ Three possible grades denoted II, III, IV

Clinical question: distinguish the grades

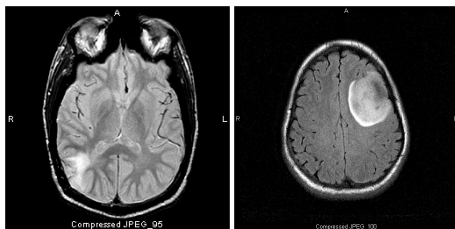


Figure: **Left:** MRI of a grade II oligodendroglioma. **Right:** MRI of a grade III anaplastic astrocytoma. Source: Manuel MSD, Steven A. Goldman

Composition:

58 tumor samples and 19 control samples

- ▶ Tumors: surgery from adult patients diagnosed with diffuse glioma.
- ▶ Histopathological types according to revised World Health Organization classification
- ▶ Control samples (n = 19): from non tumoral brain surgeries (epilepsy, benign lesion, etc.)

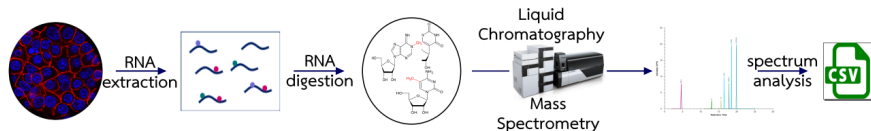
Distribution

type name number	grade-II glioma 20	grade-III glioma 20	grade-IV glioblastoma 18	control non-tumor 19
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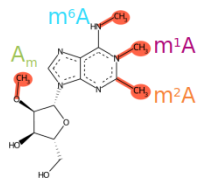
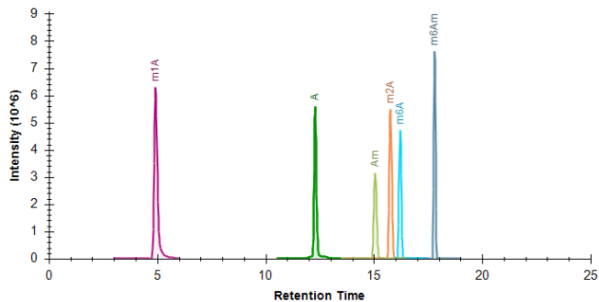
Methods - sample analysis

From tissue to data file:

1. RNA extraction (10 μ g)
2. Digestion into nucleosides
3. Liquid Chromatography Mass Spectrometry
4. Quantification (spectrum analysis)
5. Statistical & Machine Learning analysis



Mass spectrometry - spectrum of A and modified nuc



List of quantified modified nucleosides (25)

Standard	modified nucleosides
A	Am m1A m66A m66Am m6A m6Am I
C	Cm ac4C m3C m5C hm5C
G	Gm m1G m227G m27G m7G oxo8G
U	Um m3Um mcm5U mcm5s2U ncm5U Psi Queuosine

For each sample, 29 MS measures for modified and standard nucleosides.

Math: a vector of 29 dimensions \Rightarrow **epitranscriptomic profile**

Questions

1. Do epitranscriptome modification measures vary with cancer grade?
2. Can one predict glioma grade from a sample using machine learning?
3. Which combination of nucleosides are most important for prediction?

Methods

Python scripts using libraries Scikit-learn [5], Lifelines [1], Pandas [6], Matplotlib [2], Seaborn [8]

Nucleosides with decreasing quantities wrt cancer grade

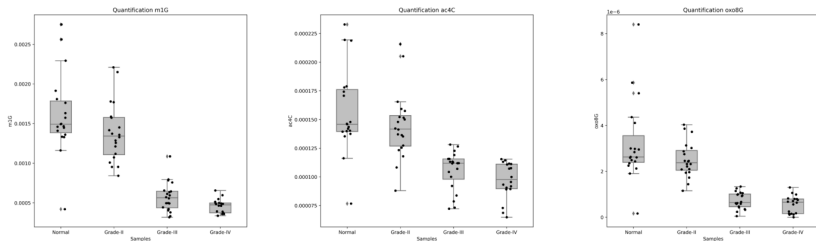


Figure: Boxplots wrt grade of m1G, ac4C, and oxo8G.

Nucleosides with increasing quantities wrt cancer grade

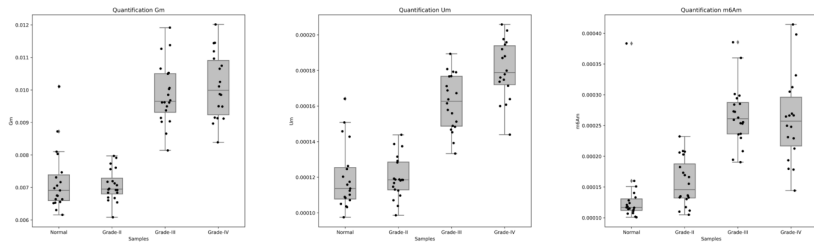
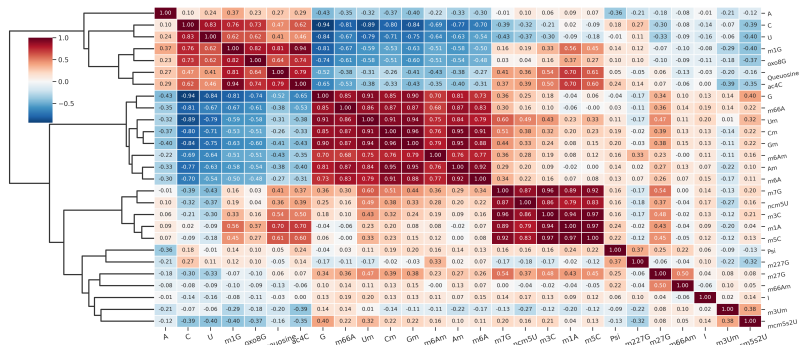


Figure: Boxplots wrt grade of Gm, Um, and m6Am.

Correlation between nucleoside quantities



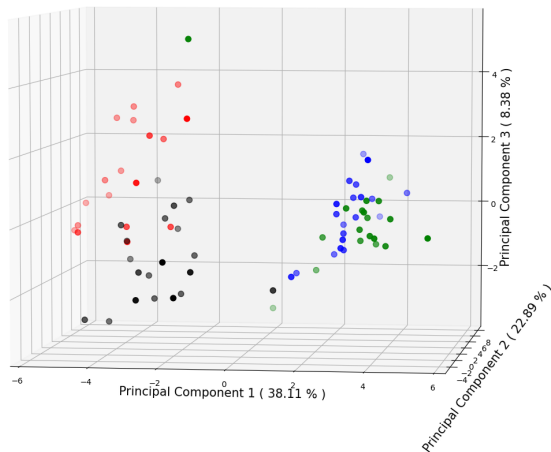
High correlations:

- ▶ Triplet: (m1A, m5C, m3C) 0.97; (Am, Cm, Gm) 0.95
- ▶ Pairs: (ac4C, m1G) 0.94; (Gm, Um) 0.93; (Am, m6A) 0.92

PCA: 3-dimensional (3D) view of PCA

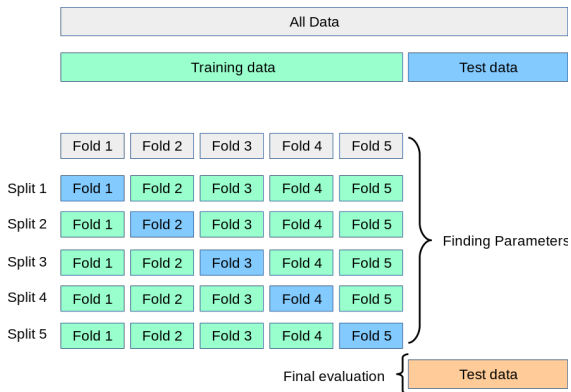
PCA using full epitranscriptomic profile: colors = grades.

GBM - 3D PCA masses normalised on SUM



Supervised Machine Learning approach

- ▶ Two steps: Learning + Evaluation; then prediction
- ▶ Different algorithms: Support Vector Machine, Random Forest, LDA, etc.



Prediction with full epitranscriptomic profile (I)

With Linear Discriminant Analysis (LDA)

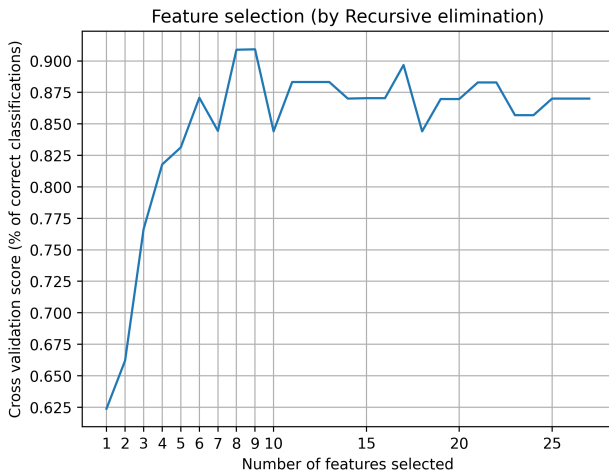
Table: Results for grade prediction with full profiles using LDA

Grade	precision	recall	f1-score	support
Grade-II	0.67	1.00	0.80	2
Grade-III	1.00	0.83	0.91	6
Grade-IV	0.88	1.00	0.93	7
Ctrl	1.00	0.80	0.89	5
weighted avg accuracy	0.92	0.90	0.90 0.90	20

Selection of most informative nucleosides for grading

Use Recursive Feature Elimination on a SVM (linear kernel):

- ▶ a profile with 9 nucleosides suffice for grade prediction
- ▶ one automatic selection yields
{Cm , Psi, Q, Um, m1G, m227G, m5C, m66A, m6Am}



1. State-of-the-art MS/MS quantifies modified nucleosides from biopsy tissue
2. Levels of **some** modified nuc. vary with diffuse glioma grades
3. Full epitranscriptomic profile allows grade prediction with 90% accuracy using machine learning approaches
4. but single nuc quantities do not!
5. Feature selection exhibits a selection of 9 nucleosides, which is sufficient for accurate prediction.

analytical
chemistry

Multivariate Analysis of RNA Chemistry Marks Uncovers Epitranscriptomics-Based Biomarker Signature for Adult Diffuse Glioma Diagnostics

S. Relier, A. Amalric, A. Attina, I.B. Koumare, V. Rigau, F. Burel Vandenbos, D. Fontaine, M. Baroncini, J.P. Hugnot, H. Duffau, L. Bauchet, C. Hirtz*, E. Rivals*, and A. David*

Cite this: *Anal. Chem.* 2022, 94, 35, 11967–11972

Publication Date: August 23, 2022

<https://doi.org/10.1021/acs.analchem.2c01526>

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Advantages:

- ▶ requires little material: 10 μg
- ▶ lasts less than 6 hours
- ▶ cheap

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Future:

- ▶ Link to known influent mutations:
IDH1/2, MGMT, BRAF, ATRX, EGFR, TERT
- ▶ Patient stratification
- ▶ Analysis from blood samples, or other fluids.
- ▶ Other cancers: breast, colorectal, pancreas.

Thanks for your attention



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Projet cofinancé par le Fonds Européen de Développement Régional

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Recall and precision

$$\text{recall} = TP / (TP + FN)$$

$$\text{precision} = TP / (TP + FP)$$

F1-score

a harmonic mean of the precision and recall (best value is 1, worse is 0).

$$F1 \text{ score} = 2 * (\text{precision} * \text{recall}) / (\text{precision} + \text{recall})$$

Accuracy

is the fraction of correct predictions over all tested samples

Principal Component Analysis: % of explained variance

First 3 components capture 69 % of variance.

