

Electrochemical and Electrochemiluminescence *in vitro* Detection Methods of a Human Cytomegalovirus in Oncology Patients

Ludmila Moranova¹, Aneta Friedl^{1,2}, Karolina Itterheimova^{1,2}, Michal Hendrych³, Martin Bartosik¹

¹ RECAMO, Masaryk Memorial Cancer Institute, Brno, Czech Republic,

² NCBR, Faculty of Science, Masaryk University, Brno, Czech Republic

³ 1st Dept. of Pathology, St. Anne's Univ. Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic

ludmila.moranova@mou.cz

Summary:

The study focuses on the detection of human cytomegalovirus (hCMV) in oncology patients, particularly in glioblastoma, and its potential role in tumor biology. Electrochemical (EC) and electrochemiluminescence (ECL) methods were developed and optimized in combination with different amplification techniques (PCR, RPA, and LAMP). The results showed that EC detection achieved a lower limit of detection than ECL, while LAMP provided the strongest signal in the shortest time. A total of 112 clinical samples were analyzed for viral DNA and RNA to identify suitable biomarkers.

Keywords: human cytomegalovirus, screen-printed carbon electrode, electrochemical bioassay, isothermal amplification technique, cancer biomarker.

Introduction

Human cytomegalovirus (hCMV) is a widespread herpesvirus proposed to exert oncomodulatory effects on several cancers by influencing cell proliferation, inflammation, and immune evasion [1]. hCMV DNA and proteins have been detected in tumors, such as glioblastoma and colorectal cancer, suggesting a possible role in tumor biology. However, detection rates in clinical samples vary dramatically from 0 to 100%, reflecting both biological heterogeneity and methodological differences [2]. Standard detection methods, such as immunohistochemistry, qPCR, and sequencing, provide good sensitivity, but each has limitations, including inconsistent antibody performance, costly instrumentation, and limited suitability for routine screening in point-of-care settings.

Methodology

Here, we address these diagnostic issues by developing new electrochemical (EC) and electro-generated chemiluminescence (ECL) bioassays that offer attractive alternatives, providing rapid, simple, and low-sample-consumption approaches that are well-suited for point-of-care testing. In this study, we optimized and compared EC and ECL bioassays coupled with vari-

ous amplification strategies, including PCR, recombinase polymerase amplification (RPA), and loop-mediated isothermal amplification (LAMP), for the detection of hCMV in real samples.

Results

First, we developed and tested three amplification strategies and combined them with EC or ECL readouts. The EC approach achieved a lower LOD and, although its linear range is narrower than that of ECL, EC appears to be a more suitable method (Fig. 1).

Amperometric EC readout combined with PCR or RPA showed high specificity, reliably distinguishing hCMV-infected from uninfected cells and demonstrating its potential for use in tumor tissue analysis. In terms of signal intensity versus amplification time, LAMP showed the best performance but suffered from nonspecific amplification. Surprisingly, the ECL assay failed to detect viral DNA under our conditions [3].

Clinical samples study

After the protocol optimization phase, a set of 112 glioblastoma IDH wild-type samples was tested for the presence of viral DNA and RNA (Fig. 2). In this study, we aim to compare different genes and their RNA products to determine which can serve as the best biomarker. DNA positivity shows the presence of the virus and

RNA can prove their activity and help with a better understanding of viral activity and its on-comodulation role in the tumor microenvironment.

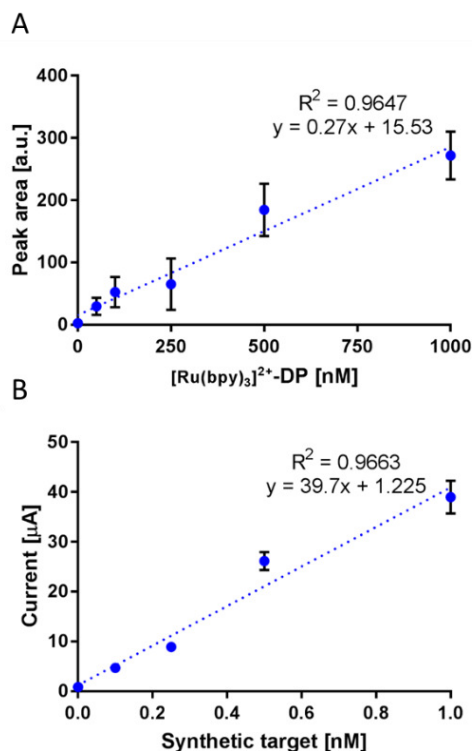


Fig. 1. Linear range of developed the methods. A: ECL of [Ru(bpy)₃]²⁺-DP measured on a photodiode with 100× amplification in the range of 1–1000 nM (LOD = 8.88 nM). B: EC of synthetic target in the linear range of 0 to 1 nM (LOD = 0.01 nM).

Our electrochemical results are compared with those obtained using a commercial kit (IVDF certified) and RT-PCR.

Conclusion

Given the specific aim of detecting viral nucleic acids rather than proteins or antibodies, diagnostic tools for oncology patients remain limited. Our results contribute to clarifying the presence and representation of hCMV in glioblastoma. To our knowledge, no previous study has systematically applied and compared a broad range of both established and newly developed technical approaches for this purpose.

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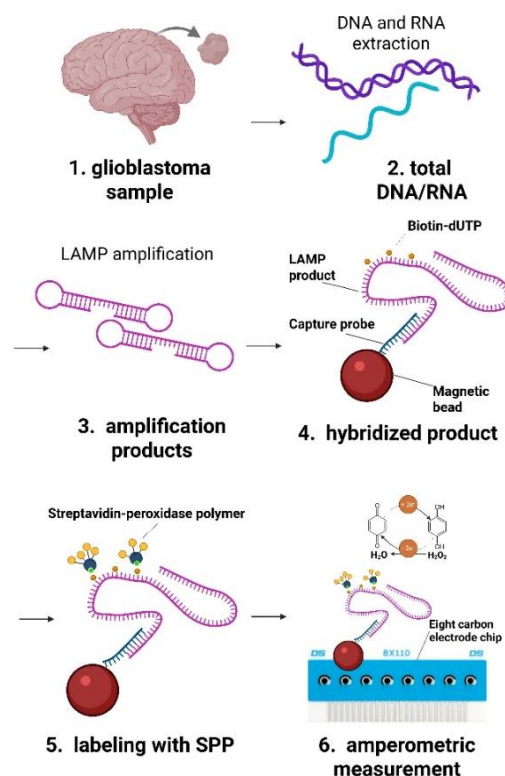


Fig. 2. Methodology scheme of the research method. (1.) Sample of glioblastoma is homogenized and used for either (2.) DNA or RNA extraction. (3.) NA is introduced into the LAMP amplification with biotin-dUTP. (4.) The amplified product with incorporated biotin-dUTP is hybridized on magnetic beads with a specific capture probe and (5.) labeled with streptavidin-peroxidase polymer. (6.) The final complex is measured on 8-electrode carbon chip with hydroquinone/hydrogen peroxide substrate.

References

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