



CHARACTERIZATION AND USE OF MONOCLONAL ANTIBODIES AGAINST BILITRANSLOCASE AND ITS DETERMINATION IN CLEAR CELL RENAL CELL CARCINOMA

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Abstract — With its worldwide incidence of about 300 000 new cases per year, clear cell Renal Cell Carcinoma (ccRCC) is the seventh most commonly diagnosed cancer in men and the ninth most commonly diagnosed cancer in women. At the hereditary and molecular levels recent research efforts describe large molecular profiling analyses for the molecular cause to be related with the development of ccRCC. Historically, von Hippel-Lindau (VHL) tumor suppressor protein located on chromosome 3p25, the loss of activity of which leads to a syndrome connected with diseases including ccRCC, was among the top genetic causes known. Monoclonal antibodies are an important tool in diagnostics and research, especially when we are dealing with a protein marker of unknown primary structure as in case of bilitranslocase (BTL). BTL is expressed on kidney cells, where it acts as an organic anion transporter. We have shown that there are differences in bilitranslocase expression in normal kidney cells versus early grade kidney cancer. A set of hybridoma cell lines producing anti-peptide monoclonal antibodies against segments 235–246 (peptide B) and 298–310 (peptide C) of predicted primary structure of bilitranslocase was cloned by limiting dilution. With a sequence of immune tests we characterized monoclonal antibodies, and used them as a tool to distinguish between grades in progression of ccRCC. We developed monoclonal antibodies against extra- (peptide B) and intra-cellular (peptide C) domains of bilitranslocase protein model. Our results are showing that these antibodies can be used in different

immunoassays. Furthermore specificity and affinity of our mAbs allowed us to assess progressive grades of clear cell renal cell carcinoma and thus introduce a potentially novel tool for the diagnostics of ccRCC.

Index Terms — ccRCC, mAb, BTL

1 BACKGROUND

Clear cell Renal Cell Carcinoma (ccRCC) is the seventh most commonly diagnosed cancer in men and the ninth most commonly diagnosed cancer in women. Von Hippel-Lindau (VHL) tumor suppressor protein located on chromosome 3p25, the loss of activity of which leads to a syndrome connected with diseases including ccRCC, was among the top genetic causes known thus far. Monoclonal antibodies are an important tool in diagnostics and research, especially when we are dealing with a protein marker of unknown primary structure as in case of bilitranslocase (BTL). BTL is expressed on kidney cells. We have shown that there are differences in bilitranslocase expression in normal kidney cells versus early grade kidney cancer. A set of hybridoma cell lines producing anti-peptide monoclonal antibodies against segments 235-246 (peptide B) and 298-310 (peptide C) of predicted primary structure of bilitranslocase was cloned by limiting dilution. With immune tests we characterized monoclonal antibodies, and used them as a tool to distinguish between grades in progression of ccRCC. We further developed monoclonal antibodies against extra- (peptide B) and intra-cellular (peptide C) domains of bilitranslocase protein model. Our results are showing that these antibodies can be used in different immunoassays. Furthermore specificity and affinity of our mAbs allowed us to assess progressive grades of clear cell renal cell carcinoma and thus introduce a potentially novel tool for the diagnostics of ccRCC.

2 OBJECTIVES

1. To obtain a novel generation of mAb against extracellular domain (peptide B) and intracellular domain (peptide C).
2. To extensively test novel mAb-producing cell lines for their antibody production capacity, clone viability, proteolysis effect on Ab stability, storage stability of mAb (freezing/thawing) (mAbs against peptides A, B, C).
3. To extensively test mAb affinity and specificity by immune methods.
4. To test a selected mAb in context of cell (immunocytochemistry-ICC) and tissue (immunohistochemistry - IHC) to assess potential diagnostic value of mAb.

3 APPROACH & METHODS

General approach

Development, characterization, production and testing of mouse mAbs for research and in vitro diagnostic procedures.

Methods

- Immunization of mice by antigens corresponding to three segments of a predicted protein sequence of bilitranslocase (65-75 (peptide A), 235-246 (peptide B) and 298-310 (peptide C).
- Production of hybridoma cell lines by fusion of mouse splenocytes and myeloma cell lines.
- Cloning of hybridoma cell lines by limiting dilution to obtain monoclonal antibody producing cell lines
- Testing of cell lines for antibody production capacity, clone viability, proteolysis effect on Ab stability, storage stability of mAb (freezing/thawing)
- Testing mAb affinity and specificity by immune methods (ELISA, dot-blot, WB)
- Testing a selected mAb in context of cell (immunocytochemistry) and tissue (immunohistochemistry) to assess its potential diagnostic value

4 RESULTS

Using hybridoma technology and limiting dilution mouse mAbs against peptides A, B, C of a predicted protein sequence of bilitranslocase were produced and characterized.

Btl expression was confirmed in biological samples (plant and mammal) using immune-assays ELISA, dot-blot, WB, ICC and IHC.

Btl expression was found by a-B mAb in N2a (mouse neuroblastoma) cells and UOK171 (clear cell renal cell carcinoma grade IV - ccRCC) cell line (Figure 1).

Btl expression was found by a-C mAb in HUVEC (human umbilical vein endothelial cells), SHSY5 (human neuroblastoma), N2a (mouse neuroblastoma) and UOK171 cells by ICC (Figure 1).

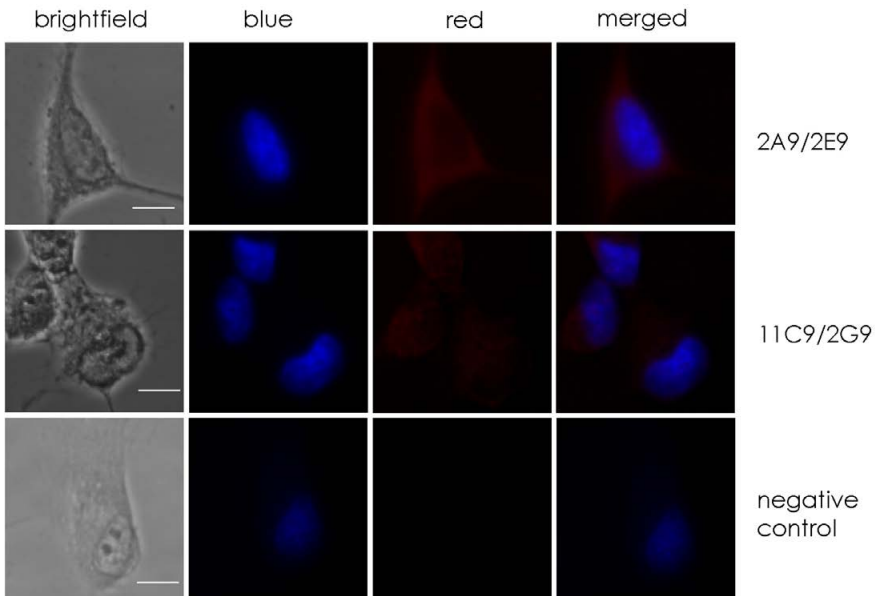


Figure 1: ICC of Btl expression (red signal) in UOK171 cell line demonstrated by a-C mAb 2A9/2E9 (first row) and by a-B mAb 11C9/2G9 (second row). Bottom row – negative control. Blue stain – nuclei.

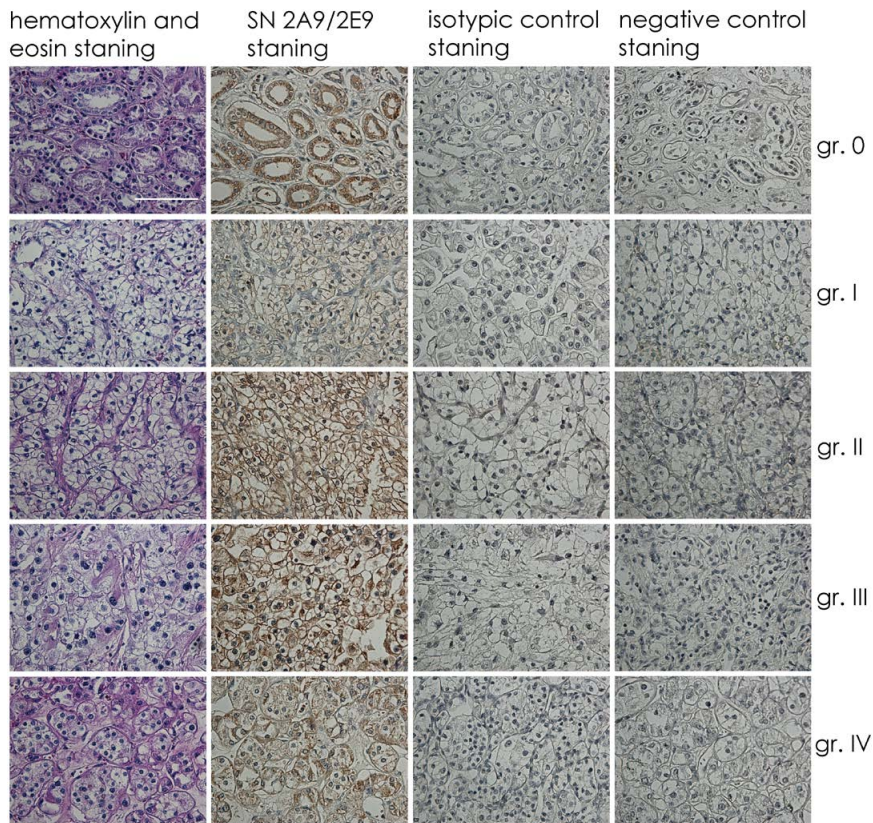


Figure 2: IHC of Btl expression in 4 progressive grades of ccRCC vs normal kidney tissue (gr. 0). First column – H&E. Second column - a-C mAb 2A9/2E9 staining. 3rd and 4th columns, negative controls. Finally, Btl expression was found by IHC stain with a-C mAb in ccRCC (Figure 2). By means of Btl expression, we cannot differentiate normal kidney from low grade tumors. However, we can differentiate normal kidney from high grade tumors and among different tumor grades (Figure 3).

5 POTENTIAL NEW PRODUCTS & SERVICES

Product: Development of an in vitro diagnostic device (IVD) based on mouse a-Btl mAb, when the Btl and Btl-like protein sequence is finally discovered.

Development of novel in vitro diagnostic device (IVD) based on patented a-prion mAb (Patent: Čurin Šerbec, V. Antibodies capable to selectively detect prion Prp Sc isoforms. 14.12.2005. München: European Patent Office)

Service: Custom made highly specific mouse mAb and human recombinant mAb.

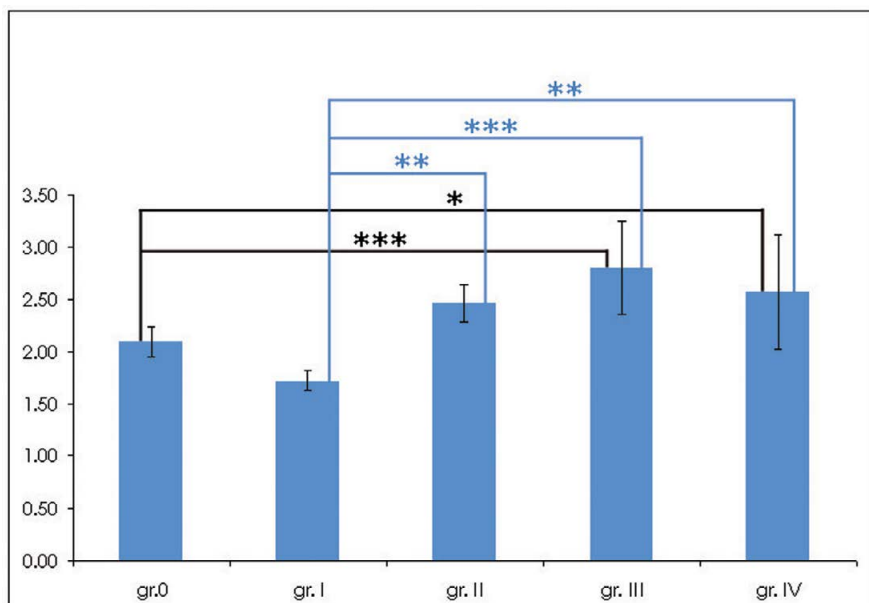


Figure 3: A histogram showing Btl expression differences in progressive ccRCC grades vs. normal kidney using IHC with a-C mAb 2A9/2E9. By means of Btl expression, we cannot differentiate normal kidney from low grade tumors.

6 CURRENT COLLABORATIONS

6.1 With other researchers and hospitals

The Universities of Udine (PP8, Trans2Care), Nova Gorica (PP3, Trans2Care), Trieste (LP, Trans2Care), Ljubljana (Faculty of Medicine, Institute of Pathology; University Medical Center in Ljubljana (Obstetrics and gynecology), Izola General Hospital.

7 CONTACT OR COLLABORATIONS NEEDED

Future collaboration with clinical laboratories is needed.

8 COMMUNICATION TOOLS

- The high level of expertise in the field of laboratory medicine as well as the high level of applicability, quality and performance of the presented methods is disseminated through the high quality scientific publications.

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): 60.000 €

9.2 For applied research (solutions for real-world problems): 60.000 €

9.3 For pilot & demonstrator activities (to develop a prototype): 200.000 €

10 CONCLUSION

Monoclonal antibodies are an important tool in diagnostics and research, especially when we are dealing with a protein marker of unknown primary structure as in case of bilitranslocase (BTL). We developed monoclonal antibodies against extra- (peptide B) and intra-cellular (peptide C) domains of bilitranslocase protein model. Our results are showing that these antibodies can be used in different immunoassays. Furthermore specificity and affinity of our mAbs allowed us to assess progressive grades of clear cell renal cell carcinoma and thus introduce a potentially novel tool for the diagnostics of ccRCC. However, a lag in basic research (sequencing of BTL protein) sheds a doubt on these results, hinders publications in high profile scientific literature as well as development of validated tests for the assessment of BTL expression in clinical samples.

ACKNOWLEDGEMENT

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