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Carbonic anhydrase IX in renal cell carcinoma: a relevant biomarker and promising therapeutic target

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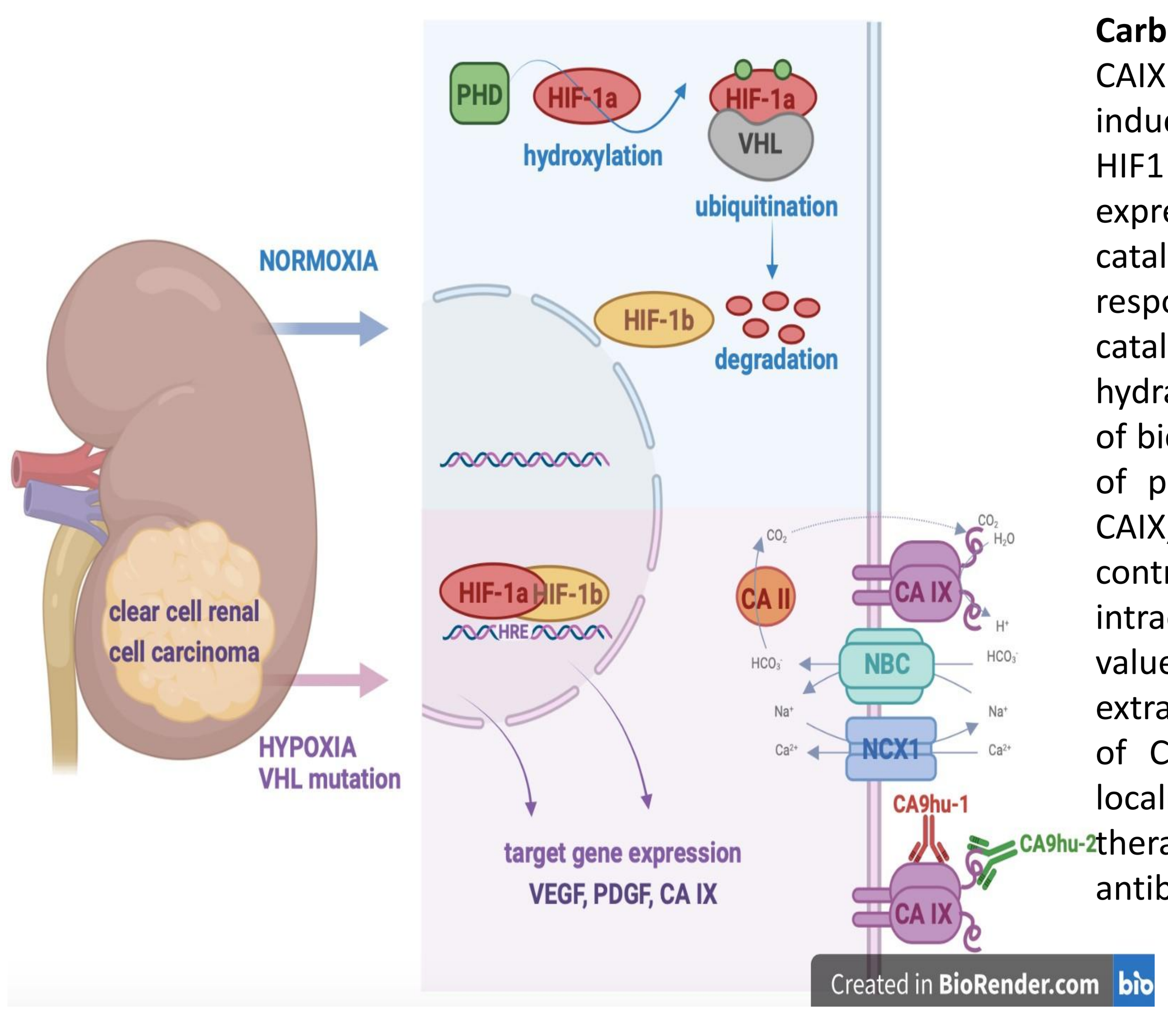
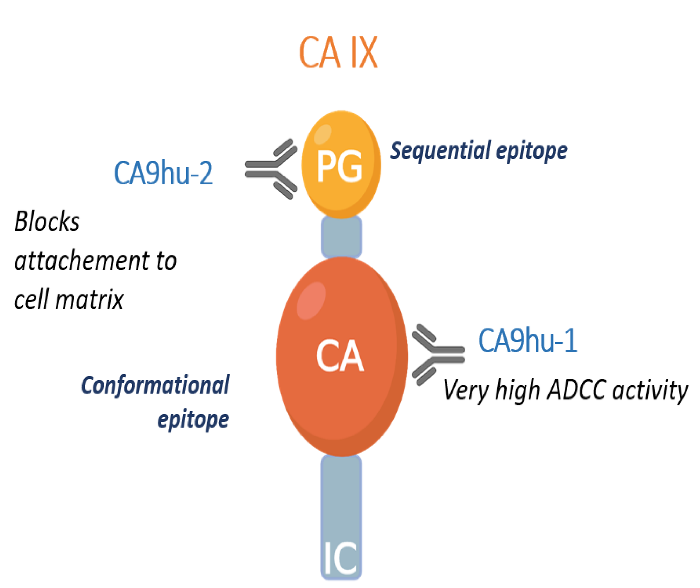
Introduction & Objectives:

Renal cell carcinoma (RCC) is one of the most aggressive urological malignancies. Despite undeniable improvements in diagnostic management and treatment strategies, the search for new therapeutic targets and approaches is a challenge at every stage of the disease. Carbonic anhydrase IX (CA IX), a cancer associated protein, is a commonly used biomarker crucial in a precise diagnosis of RCC subtypes with high positivity in clear cell RCC (ccRCC). CAIX is one of the best markers of tumor hypoxia, its expression is associated with the aggressive tumor phenotype, metastatic tendency and poor response to therapy. Expression of CAIX is principally driven by HIF1 pathway. In RCC, a high expression of CAIX is associated with VHL inactivation. CAIX contributes to the maintenance of intracellular pH within physiological values and to the acidification of extracellular space. CAIX also represents a suitable therapeutic target through specific humanized antibodies with antibody dependent cell cytotoxicity (ADCC) (Zatovicova et al., 2022). For testing new therapeutics, their combination, as well as for advanced patient stratification, it is essential to use a 3D model that most faithfully reflects the tumor tissue with inclusion of immune cells.



Carbonic anhydrase IX (CAIX)

CAIX is transmembrane hypoxia-inducible and tumor-associated protein. HIF1 is the main driver of CAIX expression in tumor tissue. The external catalytically active part of CAIX is responsible for the reversible catalyzation of CO_2 into bicarbonate by hydration. Transmembrane reabsorption of bicarbonate leads to an accumulation of protons in the extracellular space. CAIX, as a part of transport metabolon, contributes to the maintenance of intracellular pH within physiological values and to the acidification of the extracellular space. Due to the high level of CAIX in ccRCC and its membrane localization, CAIX represents a potent therapeutic target for monoclonal antibody-based therapy.

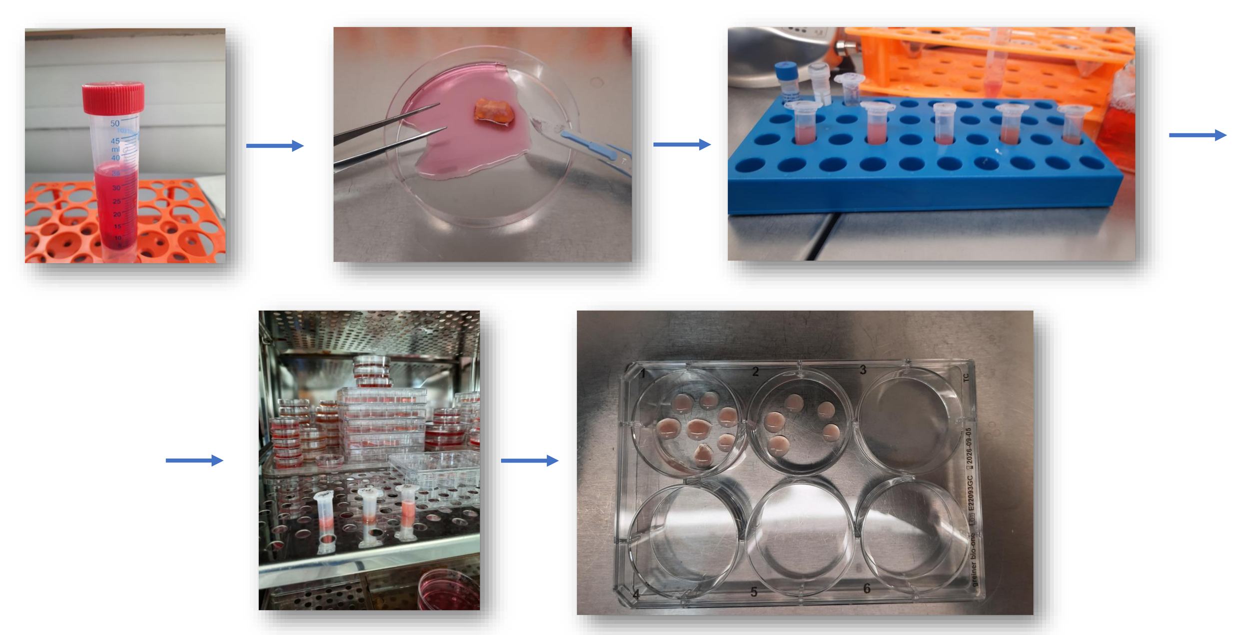


CAIX-specific humanized antibodies

Humanized antibody **CA9hu-1** recognizes a conformational epitope in the catalytic domain of the CAIX protein and activates ADCC.
Humanized antibody **CA9hu-2** recognizes a sequence epitope in the proteoglycan-like domain of the CAIX protein.

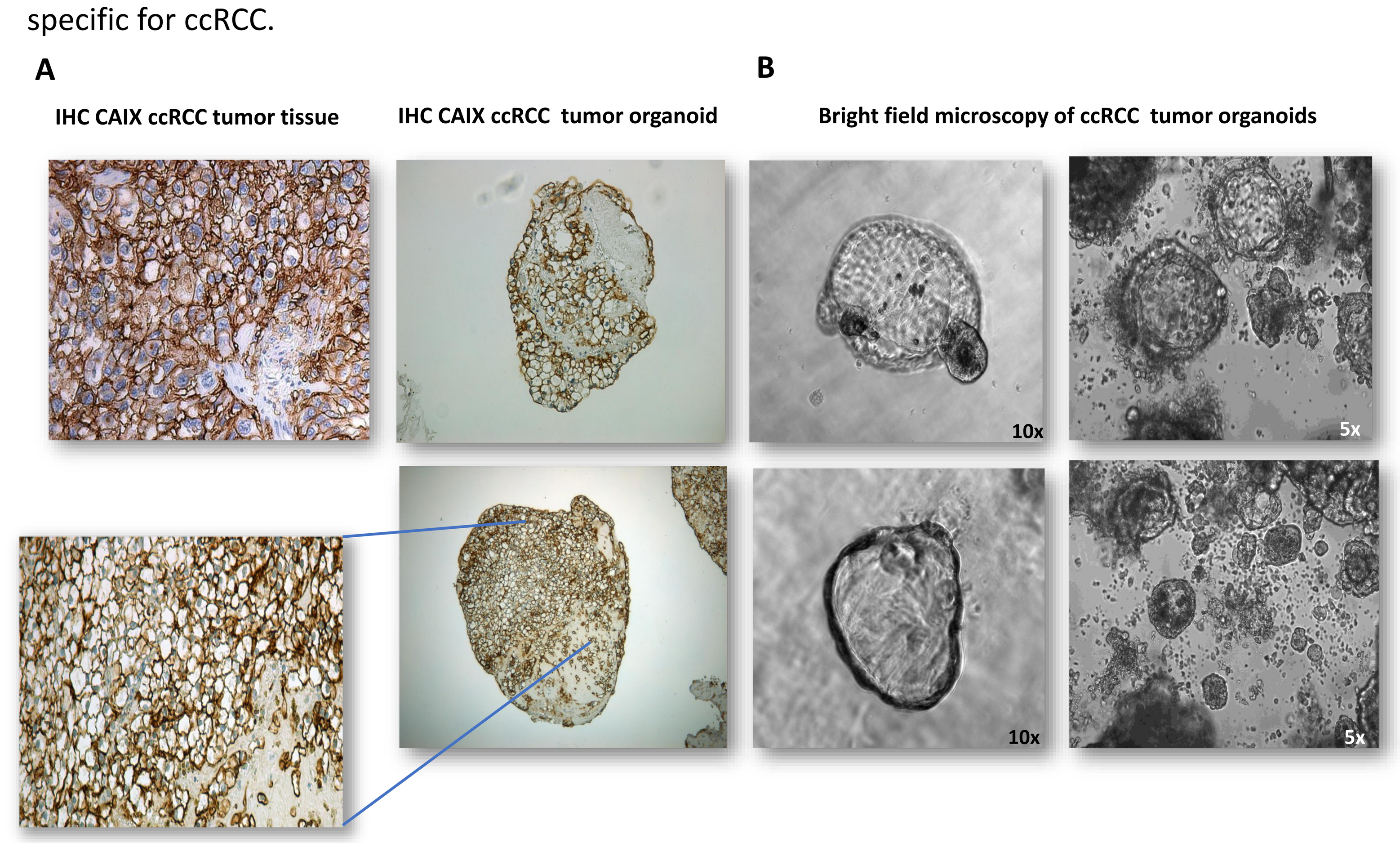
Material & Methods:

Native RCC tumor tissues, adjacent normal kidney tissues and corresponding peripheral blood were collected after partial or radical nephrectomy in the University Hospital in Bratislava. We formed organoids from RCC and normal kidney tissue after mechanical and enzymatic disruption. Peripheral blood mononuclear cells (PBMCs) were isolated from the corresponding peripheral blood. Formed organoids were subsequently co-cultured with autologous PBMCs.



Formation of ccRCC organoids.

Tumor organoids represent an effective 3D culture system mimicking tumor microenvironment and spatial architecture of tumor tissue. The patient's tumor tissue is mechanically and enzymatically disaggregated. After the separation of cancer cells, they are cultured in the presence of base membrane extracts (extracellular matrix) in a culture medium with growth factors and components specific for ccRCC.

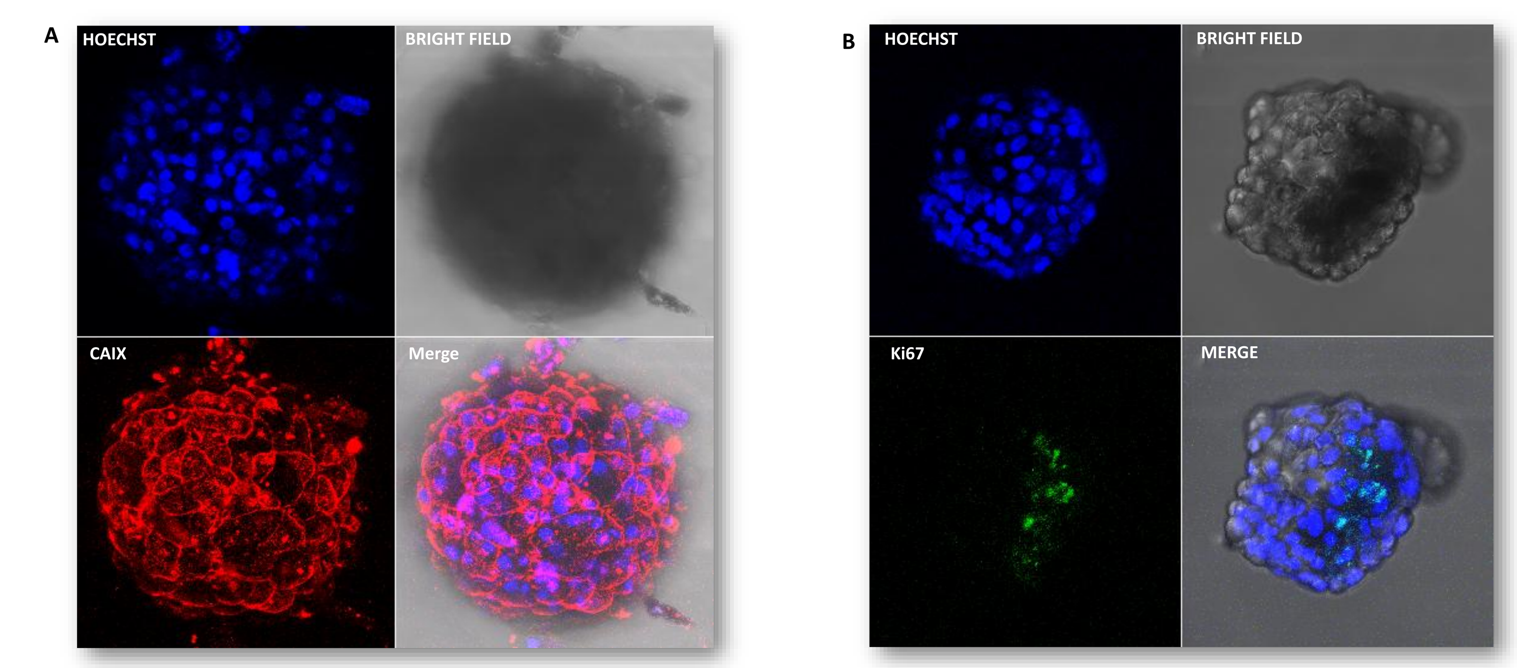


A. Immunohistochemical analysis of CAIX protein level in ccRCC tumor tissue and corresponding tumor organoid after 14 days of cultivation.
B. Morfology of ccRCC organoids – transmitted light microscopy

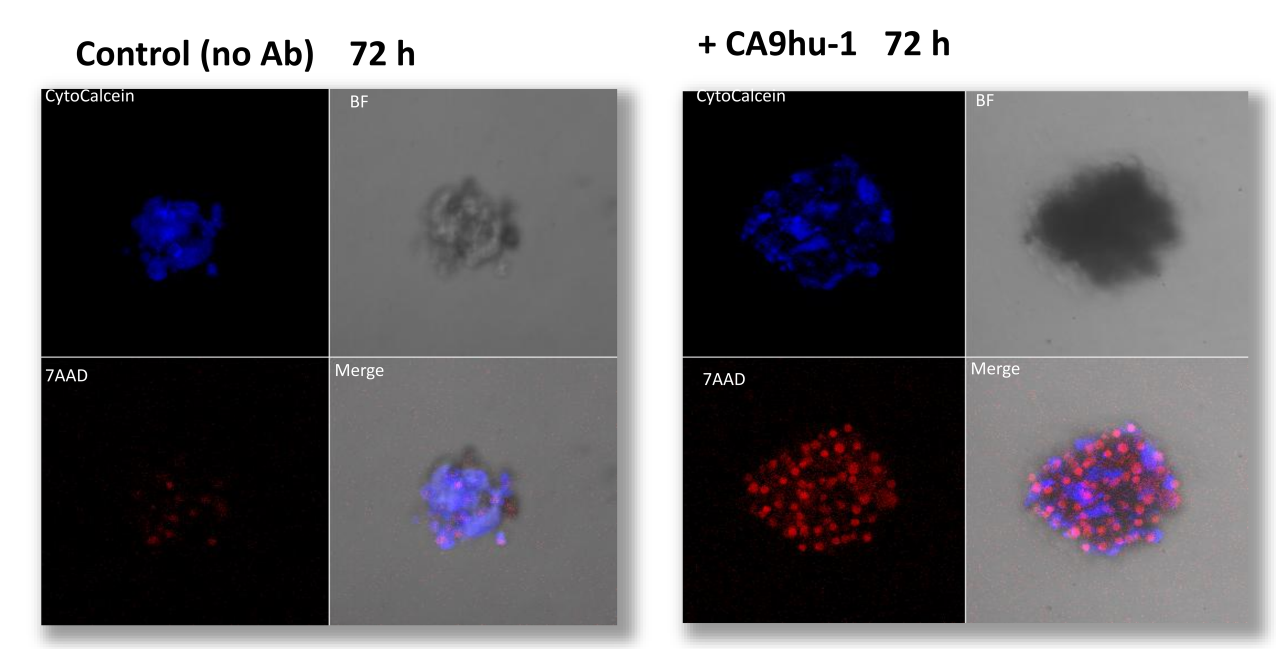
Results:

We established an effective 3D culture system for generating kidney cancer organoids from clinical samples. Of 20 samples, 70% were ccRCC, 20% papillary RCC and 10% mucinous subtype. Tumor organoids preserved the histological architectures and intra-tumoral heterogeneity comparable to tumor tissue. High expression of CAIX was also preserved in tumor organoids, organoids from normal kidney did not express CAIX. Co-cultivation of organoids with autologous PBMCs showed the penetration of immune cells inside the organoids and confirmed the immunogenicity of tumor organoids as it is also in RCC.

Conclusion: RCC organoids co-cultivated with autologous immune cells represent a valuable pre-clinical model for testing the effectiveness of new anticancer drugs as well as a new and modern approach in personalized/precision medicine.



A. Confocal microscopy of CAIX protein in living ccRCC organoids. Strong CAIX membrane positivity (red – CAIX protein, blue – nuclei)
B. Confocal microscopy of Ki67 in living ccRCC organoids (green – Ki67, blue – nuclei)



Cytotoxic effect of humanized antibody CA9hu-1 in ccRCC organoids after 72 h treatment in the presence of autologous immune cells (PBMCs). Confocal microscopy of necrotic cells (red). Preclinical efficacy testing of the humanized CA9hu-1 antibody.