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The Cancer Control Challenge:
Better Understanding It to Better Face It

# GENERATION AND CHARACTERIZATION OF A NOVEL IgY ANTIBODY AGAINST CLAUDIN 18.2 (*CLDN18.2*) FOR TRANSLATIONAL APPLICATIONS IN GASTRIC ADENOCARCINOMA

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**Introduction:** Gastric cancer is among the malignancies with the highest mortality rates, particularly in advanced stages. Loss of epithelial polarity, frequently associated with altered expression of tight junction proteins such as claudins, plays a key role in tumor progression. Claudin 18.2 (*CLDN18.2*) has gained attention due to its selective expression in gastric mucosa, and its presence in tumors combined with its absence in most normal tissues makes it a promising target for diagnostic and therapeutic strategies in oncology. **Objectives:** To obtain, characterize and validate an IgY antibody specifically targeting claudin 18.2 (*CLDN18.2*) through immunization with the synthetic PCNI peptide, evaluating its affinity and expression in gastric adenocarcinoma cell lines, with a focus on diagnostic and therapeutic applications in gastric cancer. **Methods:** Adult White Leghorn hens were immunized with the synthetic PCNI peptide, corresponding to the extracellular loop of *CLDN18.2*, combined with Montanide adjuvant, in three doses administered on days 0, 14 and 21 (CEUA Ethics Protocol No. 1326191222). Eggs were collected daily before and after immunization. IgY antibodies were extracted from egg yolks, purified using Amicon® filters and quantified. Protein integrity was assessed by SDS-PAGE, and immune response was evaluated by Dot Blot, Western Blot and ELISA. In the ELISA assay, the affinity of anti-*CLDN18.2* IgY was compared with a commercial polyclonal anti-CLDN18 antibody used as a positive control. Antibody specificity was evaluated by immunofluorescence in three gastric cancer cell lines (MKN45, ACP03 and AGP01). Statistical analyses were performed using ANOVA followed by Tukey's post hoc test in GraphPad Prism 5.5 and R software (version 4.4), with $p \leq 0.05$ considered statistically significant. **Results:** Purification using Amicon columns resulted in a recovery rate above 60% while preserving antibody structural integrity, as confirmed by SDS-PAGE, which revealed distinct bands corresponding to the light (~27 kDa) and heavy (~67 kDa) chains.

Dot Blot analysis demonstrated a progressive increase in immune response throughout the immunization period, and Western Blot confirmed antibody specificity and integrity. ELISA assays indicated strong affinity of the anti-claudin 18.2 IgY antibody for the PCNI peptide, corresponding to the extracellular domain of the protein, with concentrations of 1395 µg/mL and 2739 µg/mL showing statistically significant differences compared to the positive control ($R = 0.98$; $p < 0.0001$). Immunofluorescence assays confirmed the expression and specificity of the antibody in vitro, showing selective staining in all tested cell lines (MKN45, ACP03 and AGP01) and absence of signal in negative controls. Compared with the commercial polyclonal anti-CLDN18 antibody, the produced anti-claudin 18.2 IgY antibody exhibited equivalent reactivity, validating its functionality in both metastatic gastric cancer cell lines (MKN45 and AGP01) and primary tumor cells (ACP03). **Conclusion:** This study demonstrates the feasibility of producing IgY antibodies against *CLDN18.2* in a scalable and cost-effective manner. This represents the first reported production of an IgY antibody targeting the *CLDN18.2* isoform, showing high specificity and strong potential for diagnostic and therapeutic applications in gastric cancer.

**Keywords:** Gastric cancer; Claudin 18.2; Diagnostic biomarker; IgY antibody.