Analysis of “Electrochemical immunosensor for IL-13 Receptor a2 determination and discrimination of metastatic colon cancer cells (Valverde et al.)”

Scientific Poster on Analysis of Research Knowledge

Presented by Heeae Noh, Seo Jin Lee, and Yeongbi Son

Overview

The paper proposes the first-ever researched electrochemical immunosensor that targets IL-13 receptor. IL-13 receptor is a pleiotropic immune regulatory cytokine, and this research paper specifically focuses on IL-13Ra2, which is highly expressed in late-stage cancer and is closely related to colon cancer metastasis. The immunosensor is based on sandwich-type assay and amperometric detection using SPCE. The results show the immunosensor with high sensitivity and selectivity.

Figure 1

IL-13Ra2 Immunosensor

- Sandwich (ELISA) Assay

  Sandwich assay is characterized by 3 main materials: primary antibody, target protein, labeled secondary antibody. Combinant of these 3 materials is said to look like a sandwich. The primary antibody conjugates on the surface of the target protein. Then, the labeled secondary antibody conjugates with the primary antibody. By detecting the labeling attached to the secondary antibody, the amount of target protein could be deduced.

  In the case of IL13Ra2 Immunosensor, the surface that the primary antibody conjugates is EDC/sulfo-NHS activated HOOC-magnetic bead. The primary antibody is anti-IL13sRa2 (CAb) antibody, and the secondary antibody is biotinylated secondary antibody (BDAb), which is labeled with Strep-HRP. The researchers used raw lysates, so instead of CAb conjugating with IL13Ra2 on the surface of the cell, CAb conjugates on the MB so that the freely-floating IL13Ra2 can conjugate on CAb. BDAb completes the sandwich by conjugating with IL13Ra2.

Amperometric Detection

- SPCE Reaction

  After the sandwich assay is completed, the expression of IL13Ra2 is detected via amperometric measurement of hydrogen peroxide as a way to visualize IL13Ra2 expression.

  When IL13Ra2 attaches on the MB, thereby attaching on the immunosensor, benzoquinone (BQ), the oxidized form of the mediator, receives two electron and gets reduced to hydroquinone (HQ). Then, HQ is re-oxidized by HRP to BQ, which also results in the reduced form of HRP. The reduced form of HRP then oxidizes hydrogen peroxide (H2O2), resulting in water (H2O) and oxidized form of HRP. The cycle is repeated as HQ regenerates HRP.

  When there are more IL13Ra2 present, more reaction occurs in a relative time, which means more H2O2 are oxidized into H2O. The immunosensor detects the cathodic current as H2O2 reduces to H2O over a set amount of time. The amount of IL13Ra2 present and the cathodic current produced with the enzymatic reduction of H2O2 is directly proportional.

Table 2

The table shows the values for specific variables that allows the optimization of the immunosensor to be faster and more accurate. The variables include the amount of HOOC-MBs, concentration of CAb and BDAb, incubation times for each step, and the dilution value of Strep-HRP.

Graph 3

a) The linear calibration plot shows the relationship between the concentration of IL13Ra2 and the amperometric measurement. As the concentration of IL13Ra2 increases, more cathodic current can be detected to the increased amount of H2O2 reduction.

b) The graph is constructed to show the increase in the amperometric measurement as it goes down. The numbers on the right of the lines represent the concentration of IL13Ra2, which also increases as the graph goes down. Starting with 50 nA, with 5 seconds of reaction, the graph displays the relationship between the concentration of IL13Ra2 and cathodic current detected, which is directly proportional.

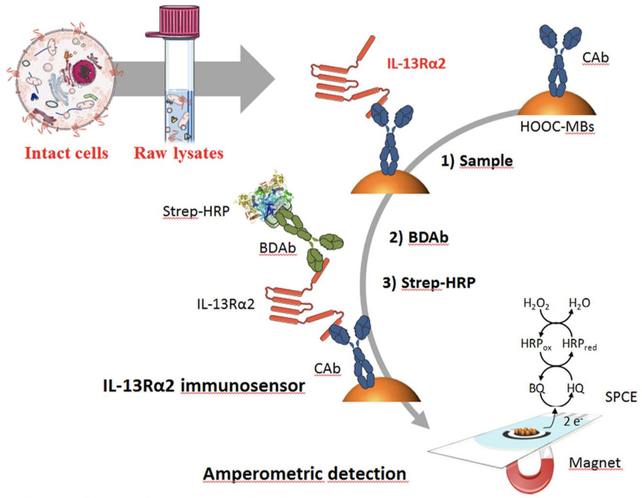
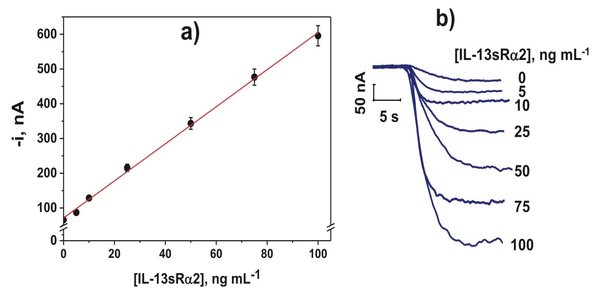


Figure 1. Display of the developed IL-13Rα2 sandwich immunosensor

Optimization of the different experimental variables involved in the immunosensor developed for the amperometric determination of IL-13sRα2.

Variable	Checked range	Selected value
HOOC-MBs, μL	–	3.0
[CAb], μg mL^{-1}	0.0 – 25.0	10.0
Incubation time CAb, min	15 – 90	60
Incubation time IL-13sRα2, min	15 – 60	30
[BDAb], μg mL^{-1}	0.25 – 2.5	0.5
Incubation time BDAb, min	15 – 60	30
Strep-HRP dilution	1/250 – 1/10000	1/5000
Incubation time Strep-HRP, min	15 – 60	15

Table 2. Optimization of the different experimental variables



Graph 3. Calibration plot constructed with HRP-Strep-BDAb-IL-13sRα2-CAb-MBs/SPCE immunosensor

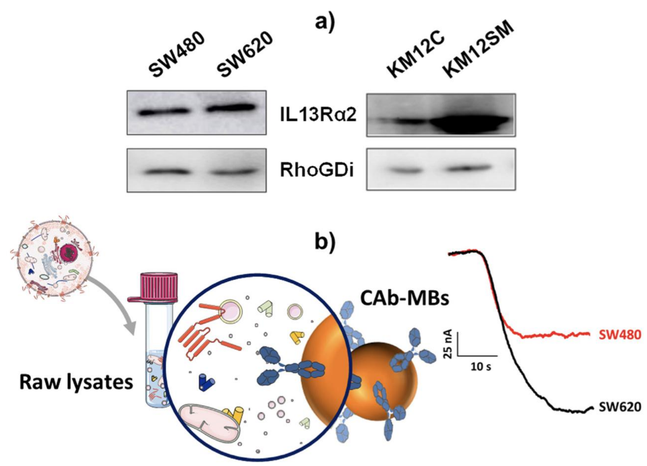


Figure 4. a) Assessment of IL-13Ra2 status in cancer cells by Western blot
b) Amperometric measurements provided by the immunosensor for cell lysates

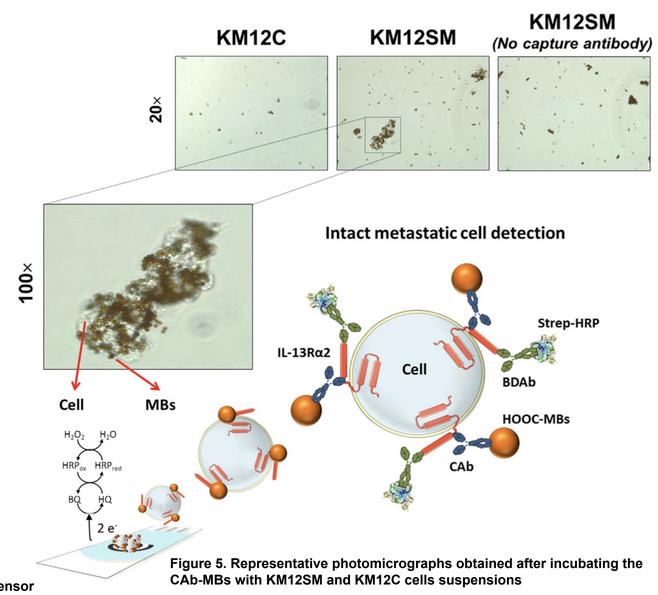


Figure 5. Representative photomicrographs obtained after incubating the CAb-MBs with KM12SM and KM12C cells suspensions

Conclusion

The IL-13Ra2 Immunosensor is the first immunosensor that detected a IL13Ra2, a biomarker of emerging relevance in metastatic colon cancer.

This immunosensor, involving a sandwich assay implemented onto the surface of MBs and amperometric detection at SPCEs, exhibits higher sensitivity and selectivity.

Also, it allows the determination of the IL-13Ra2 content in small amounts of cell lysate and obtains the result without damages on membrane surface. The immunosensor measures and discriminates between colon cancer cells regarding their metastatic potential.

This research is expected as a starting point for developing routine metastatic colon cancer detection device with high portable and point of care.

Our Thoughts & Questions

- What is the control variable of western blot? Why didn’t the research team attempt western blot or the immunosensing over the normal cells?
  - There must have been a picture of the western blot result with non-cancerous normal cell as the control variable to prove that there are IL13Ra2 on metastatic cancer cell only, not on normal cells.
  - The team needed to check the sensor doesn’t react to the normal cells not just the non-metastatic cancer cells.
- What is HAMAs, the cause of the crosslink error in the second experience?
  - HAMAs is a human’s antibody that reacts to the mouse’s immunoglobulin. The research team concluded that the crosslinking error on the mouses’ cell is due to the link between the immunosensors’ HAMAs and the immunoglobulin, so the immunoassay on the human cells would not show that kinds of crosslink.
  - To be sure with the error, the team should have checked the tolerances between the mouses’ cells and the humans’ cells.
- What is the purpose of this immunosensor if IL13Ra2 receptors are only prominent in metastatic cancer cells of patients with advanced-stage cancer?
  - This immunosensor seems useless for prediction and early diagnosis of cancers.
  - However, this immunosensor may be the start of the technologies that allows researchers to distinguish between normal cells, cancerous cells, and metastatic cells.

Figure 4

SW620 and KM12SM are the metastatic versions of SW480 and KM12C, respectively.

- SW480 and KM12C are the cancerous human colon cells
- SW620 and KM12SM are the cancerous cells obtained from mice metastatic liver cancer.

a) Western blot analysis

Thick bands represent high expression or high injection, and vice versa. The first line of the Western blot (IL12Ra2) represents the expression of target protein IL13Ra2 on the four types of cancerous cells. The second line of the Western blot (RhoGDi) represents the amount of solution injected during the Western blot practice. This is necessary to show that equal amounts of solutions were injected into the protein membrane, hence proving that the there weren’t intentional actions during the lab to manipulate the results in favor of the purpose of the lab.

The Western blot results show that there was an obvious high expression of IL13Ra2 in the metastatic cells SW620 and KM12SM compared to their isogenic cell pairs. This agrees with the expectation that IL13Ra2 is highly expressive in metastatic cells, thus proving the point of the research valid.

b) Graph analysis

According to Graph 3, steeper line in the graph represents higher expression of the target protein IL13Ra2. Using Graph 3 as the marker, the amperometric measurement of SW480 is close to 50 nA, while the amperometric measurement of SW620 is close to 100 nA. This corresponds with the Western blot results, thus agrees with the expectation that more IL13Ra2 are expressed in metastatic cells than their isogenic pairs.

Figure 5

Observation

- The photomicrograph 20x KM12SM shows the largest brown area.
- The photomicrograph 20x KM12C and KM12SM show that among KM12 line, more antibody bound in KM12SM, which is a metastatic cell. This represents that the antibodies used in the immunosensor is specific to IL13Ra which are mostly present on the surface of metastatic cells.
- The photomicrograph 20x KM12C shows that antibody binding with cancer cells didn’t happen in the KM12C. This represents that the immunosensor is accurate, and doesn’t make binding any other receptors or biomaterials.

Explanation

- To show that antibody binding is only specific to IL13Ra2, researchers took photomicrographs of cell lysates of KM12 line {KM12C, KM12SM, KM12SM without CAb} by using optical microscope. They could have used SW line {SW620, SW480,SW480 without CAb}, but KM12 line showed higher expression of IL13Ra2, so KM12 line was used to test the specificity of the antibodies used in the immunosensor..
- Each cancer cell has multiple target protein IL13Ra2 on the surface instead of just one, so multiple CAb-MBs would bind with one cell. As the cell contains more IL13Ra2, larger amount of CAb-Mbs would bind, and the colored Mbs appear on the photograph, indicating high expression of IL13Ra2
- The brown colored material in the photomicrograph represent Mbs. If the cell’s receptors IL13Ra2 bind with the immunosensors’ CAb-MB, there might be relatively large area covered with brown color. If not, Mbs just floats around. Therefore, the cluster of colors represent cells with high IL13Ra2 expression, but there are some spots of colors in KM12C and KM12SM since there are still MBs in the solution, but not clustered together.

The image represents the process of how the immunosensor can visualize the presence of metastatic cancer cells by detecting multiple CAb-MBs attached to IL13Ra2 on the metastatic cancer cell. Each cell has more than one receptors, so more than one CAb-MBs will conjugate on the cell.