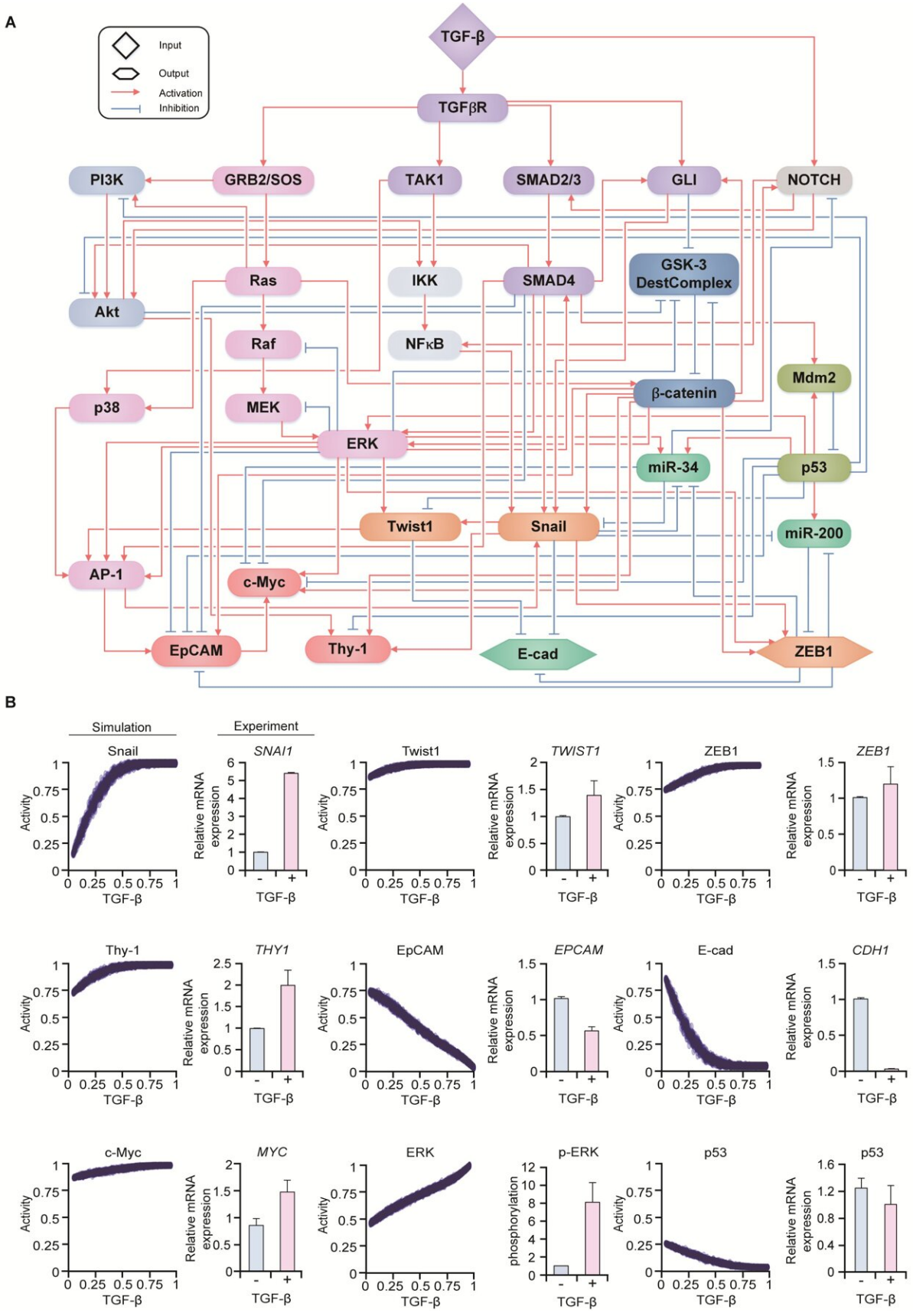
Strategy could remove metastatic traits and drug resistance from lung cancer cells

January 30 2023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E-cad
CDH1
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p53
Activity
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Figure 1. Construction of the mathematical model of the regulatory network to represent the EMT phenotype based on the interaction between various molecules related to EMT.(A) Professor Kwang-Hyun Cho's research team investigated numerous literatures and databases related to complex EMT, and based on comparative analysis of cell line data showing epithelial and mesenchymal cell conditions, they extracted key signaling pathways related to EMT and built a mathematical model of regulatory network (B) By comparing the results of computer simulation analysis and the molecular cell experiments, it was verified how well the constructed mathematical model simulated the actual cellular phenomena. Credit: *Cancer Research* (2023). DOI: 10.1158/0008-5472.CAN-22-1559

A research team led by Professor Kwang-Hyun Cho from the Department of Bio and Brain Engineering at KAIST succeeded in using systems biology research to change the properties of carcinogenic cells in the lungs and eliminate both drug resistance and their ability to proliferate out to other areas of the body.

Incidences of cancer increase within aging populations. Fatality rates are especially high when early detection does not happen in time and metastasis has occurred in various organs. In order to resolve this problem, a series of attempts were made to remove or lower the ability of cancer cells to spread, but they resulted in cancer cells in the intermediate state becoming more unstable and even more malignant, which created serious treatment challenges.

Professor Kwang-Hyun Cho's research team simulated various cancer cell states in the Epithelial-to-Mesenchymal Transition (EMT) of lung cancer cells, between epithelial cells without metastatic ability and mesenchymal cells with metastatic ability. A molecular network mathematical model was established, and key regulators that could

reverse the state of the mesenchymal cells, which had acquired invasiveness and drug resistance, back to the epithelial cells were discovered through computer simulation analysis and molecular cell experiments.

In particular, this process succeeded in properly reverting the mesenchymal lung cancer cells to a state where they were sensitive to chemotherapy treatment while avoiding the unstable EMT hybrid cell state in the middle process, which had remained a difficult problem.

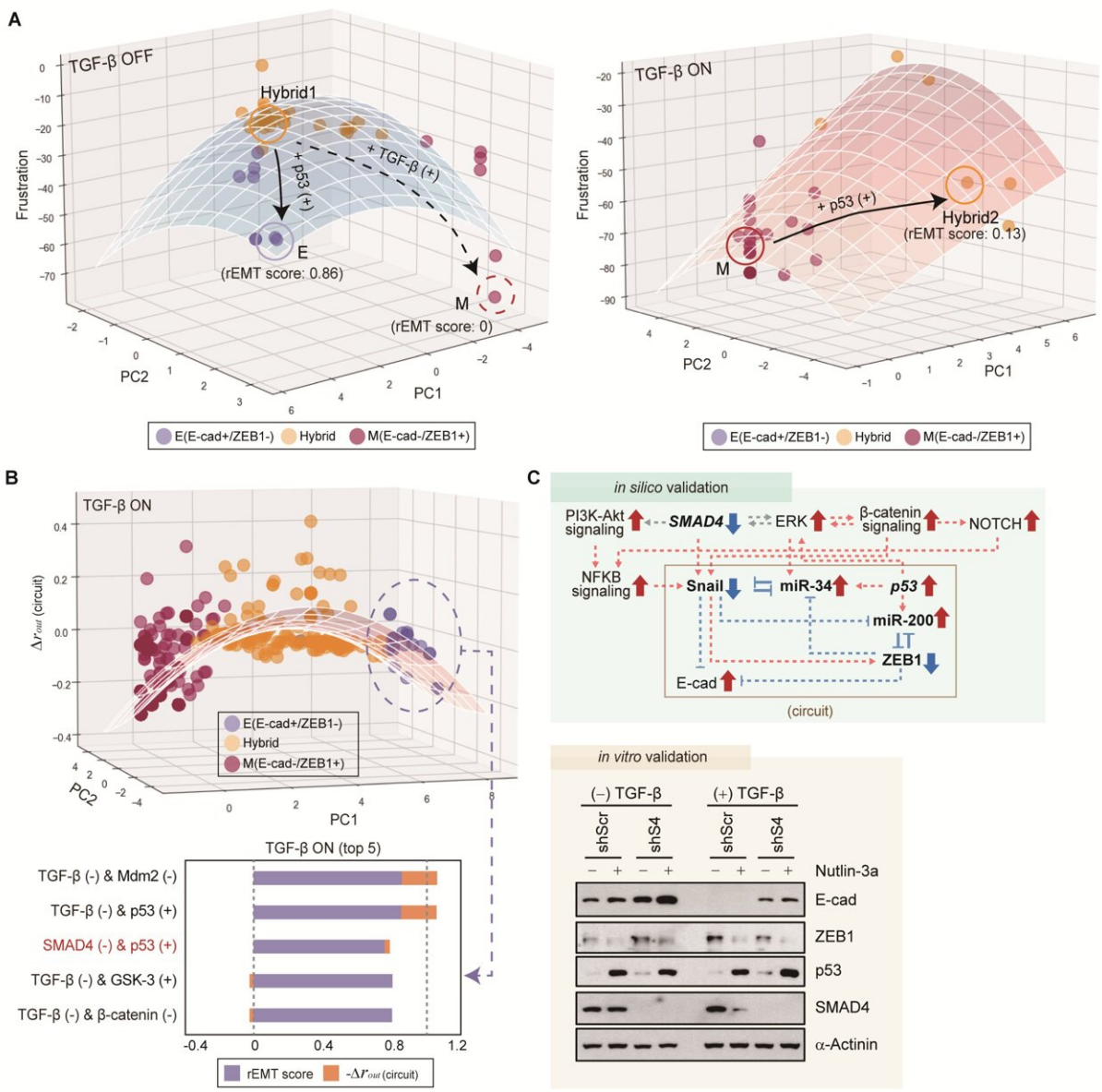

Figure 2. Understanding of various EMT phenotypes through large-scale computer simulation analysis and complex system network control technology.(A) Through computer simulation analysis and experiments, the research team found that complete control of EMT is impossible with single-molecule control alone. In particular, through comparison of the relative stability of attractors, it was revealed that the cell state exhibiting EMT hybrid characteristics has unstable properties. (B), (C) Based on these results, Prof. Cho's team identified two feedbacks (positive feedback consisting of Snail-miR-34 and ZEB1-miR-200) that play an important role in avoiding the EMT

hybrid state that appeared in the TGF-β-ON state. It was found through computer simulation analysis that the two feedbacks restore relatively high stability when the excavated p53 and SMAD4 are regulated. In addition, molecular cell experiments demonstrated that the expression levels of E-cad and ZEB1, which are representative phenotypic markers of EMT, changed similarly to the expression profile in the epithelial cell state, despite the TGF-β-ON state. Credit: *Cancer Research* (2023). DOI: 10.1158/0008-5472.CAN-22-1559

The results of this research, in which KAIST Ph.D. student Namhee Kim, Dr. Chae Young Hwang, Researcher Taeyoung Kim, and Ph.D. student Hyunjin Kim participated, have been published in the international journal *Cancer Research* on January 30th. The paper is titled "A cell fate reprogramming strategy reverses epithelial-to-mesenchymal transition of lung cancer cells while avoiding hybrid states."

Cells in an EMT hybrid state, which are caused by incomplete transitions during the EMT process in cancer cells, have the characteristics of both epithelial cells and mesenchymal cells and are known to have high drug resistance and metastatic potential by acquiring high stem cell capacity. In particular, EMT is further enhanced through factors such as transforming growth factor-beta (TGF-β) secreted from the tumor microenvironment (TME) and, as a result, various cell states with high plasticity appear.

Due to the complexity of EMT, it has been very difficult to completely reverse the transitional process of the mesenchymal cancer cells to an epithelial cell state in which metastatic ability and drug resistance are eliminated while avoiding the EMT hybrid cell state with high metastatic ability and drug resistance.

Professor Kwang-Hyun Cho's research team established a mathematical

model of the gene regulation network that governs the complex process of EMT, and then applied large-scale computer simulation analysis and complex system network control technology to identify and verify "p53," "SMAD4," and "ERK1" and "ERK 2" (collectively ERKs) through molecular cell experiments as the three key molecular targets that can transform lung cancer cells in the mesenchymal cell state, reversed back to an epithelial cell state that no longer demonstrates the ability to metastasize, while avoiding the EMT hybrid cell state.

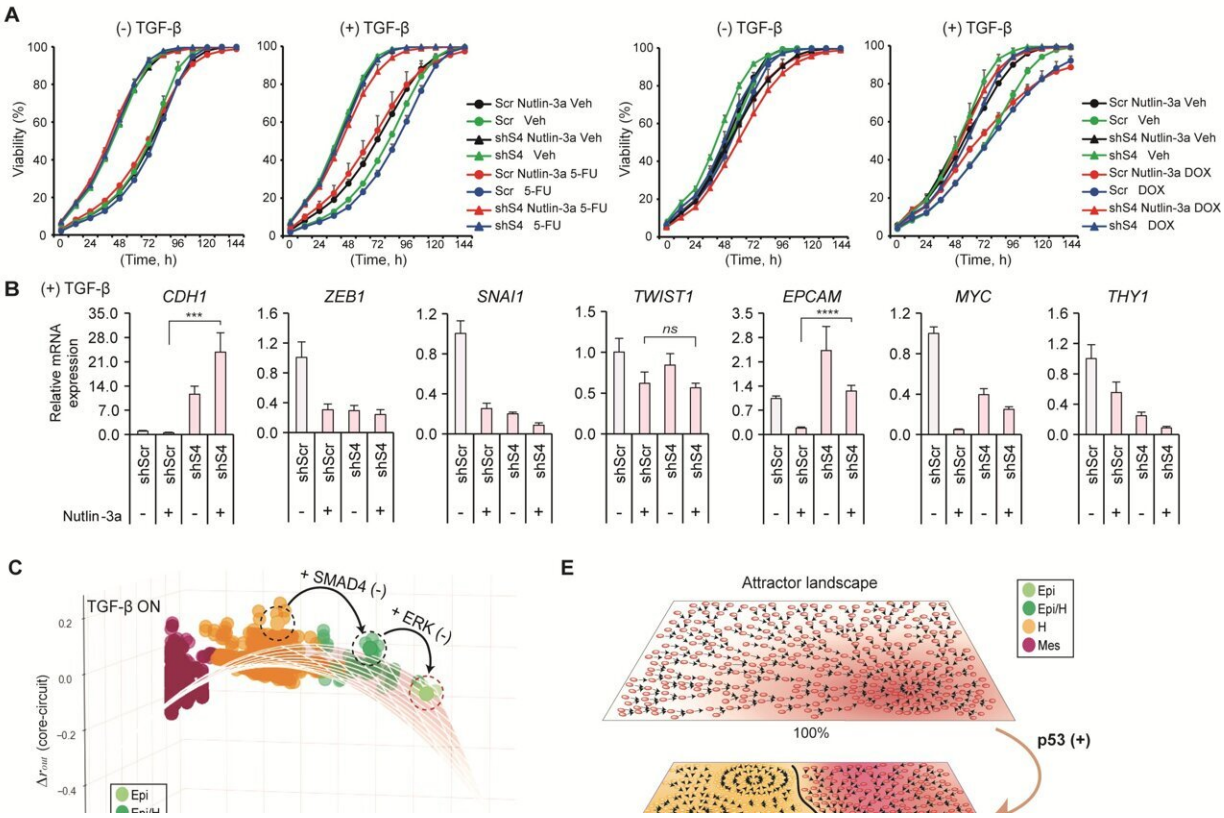

Figure 3. Complex molecular network analysis and discovery of reprogramming molecular targets for intact elimination of EMT hybrid features.(A) Controlling the expression of p53 and SMAD4 in lung cancer cell lines was expected to overcome drug resistance, but contrary to expectations, chemotherapy responsiveness was not restored. (B) Professor Kwang-Hyun Cho's research team additionally analyzed computer simulations, genome data, and experimental

results and found that high expression levels of TWIST1 and EPCAM were related to drug resistance. (C) Prof. Cho's team identified three key molecular targets: p53, SMAD4 and ERK1 & ERK2. (D), (E) Furthermore, they identified a key pathway that plays an important role in completely reversing into epithelial cells while avoiding EMT hybrid characteristics, and confirmed through network analysis and attractor analysis that high stability of the key pathway was restored when the proposed molecular target was controlled. Credit: *Cancer Research* (2023). DOI: 10.1158/0008-5472.CAN-22-1559

In particular, by analyzing the molecular regulatory mechanism of the complex EMT process at the system level, the key pathways were identified that were linked to the positive feedback that plays an important role in completely returning cancer cells to an epithelial cell state in which metastatic ability and drug resistance are removed.

This discovery is significant in that it proved that mesenchymal cells can be reverted to the state of epithelial cells under conditions where TGF-β stimulation are present, like they are in the actual environment where cancer tissue forms in the human body.

Abnormal EMT in cancer cells leads to various malignant traits such as the migration and invasion of cancer cells, changes in responsiveness to chemotherapy treatment, enhanced stem cell function, and the dissemination of cancer. In particular, the acquisition of the metastatic ability of cancer cells is a key determinant factor for the prognosis of cancer patients. The EMT reversal technology in lung cancer cells developed in this research is a new anti-cancer treatment strategy that reprograms cancer cells to eliminate their high plasticity and metastatic potential and increase their responsiveness to chemotherapy.

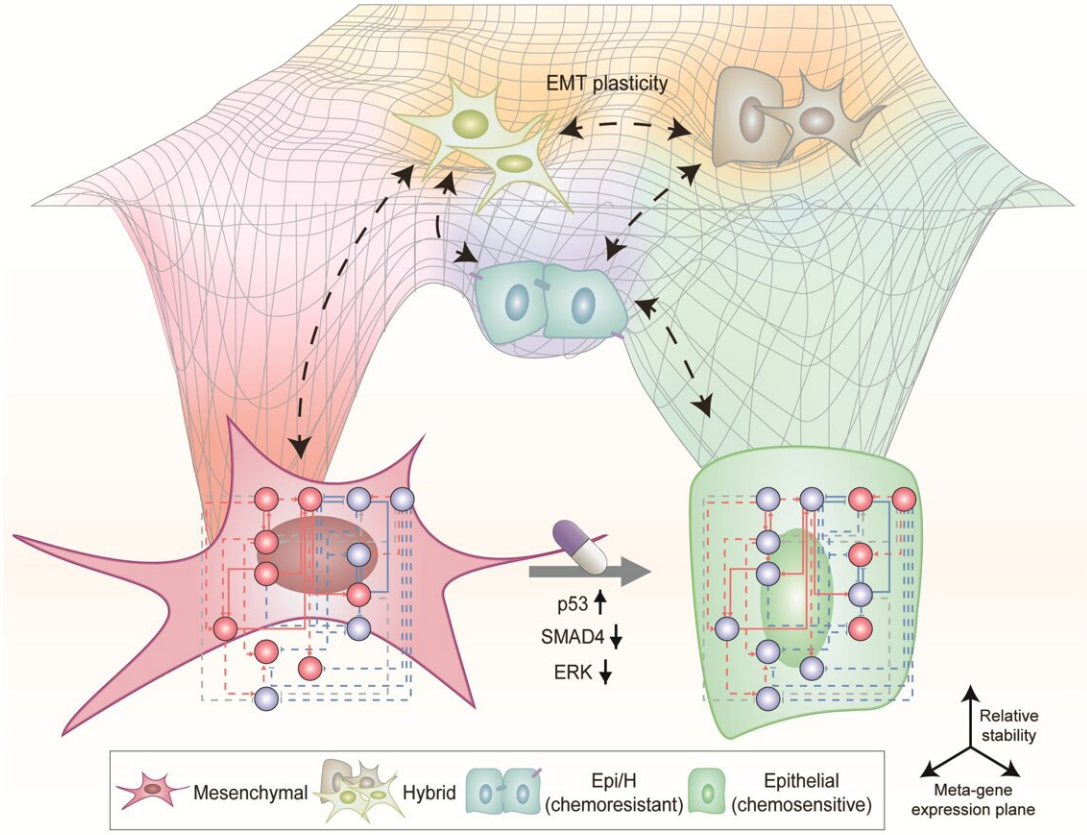

Figure 5. A schematic representation of the research results.Prof. Cho's research team identified key molecular regulatory pathways to avoid high plasticity formed by abnormal EMT of cancer cells and reverse it to an epithelial cell state through systems biology research. From this analysis, a reprogramming molecular target that can reverse the state of mesenchymal cells with acquired invasiveness and drug resistance to the state of epithelial cells with restored drug responsiveness was discovered. For lung cancer cells, when a drug that enhances the expression of p53, one of the molecular targets discovered, and inhibits the expression of SMAD4 and ERK1 & ERK2 is administered, the molecular network of genes in the state of mesenchymal cells is modified, eventually eliminating metastatic ability and it is reprogrammed to turn into epithelial cells without the resistance to chemotherapy treatments. Credit: *Cancer Research* (2023). DOI: 10.1158/0008-5472.CAN-22-1559

Professor Kwang-Hyun Cho said, "By succeeding in reversing the state of lung cancer cells that acquired high metastatic traits and resistance to drugs and reverting them to a treatable epithelial cell state with renewed sensitivity to chemotherapy, the research findings propose a new strategy for treatments that can improve the prognosis of cancer patients."

Professor Kwang-Hyun Cho's research team was the first to present the principle of reversal treatment to revert cancer cells to normal cells, following through with the announcement of the results of their study that reverted colon cancer cells to normal colon cells in January of 2020, and also presenting successful re-programming research where the most malignant basal type breast cancer cells turned into less-malignant luminal type breast cancer cells that were treatable with hormonal therapies in January of 2022.

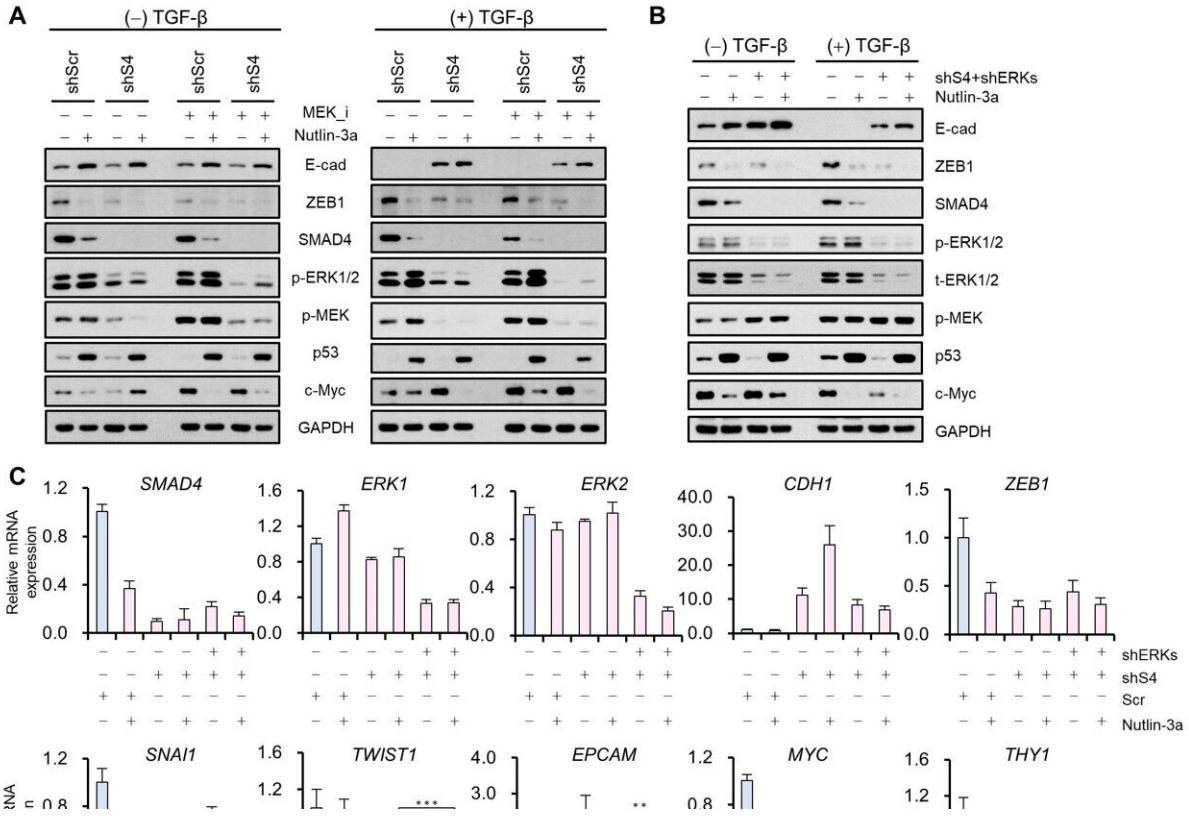

Figure 4. Verification through experiments with lung cancer cell lines.When p53 was activated and SMAD4 and ERK1/2 were inhibited in lung cancer cell lines, (A), (B) E-cad protein expression increased and ZEB1 protein expression decreased, and (C) mesenchymal cell status including TWIST1 and EPCAM and gene expression of markers related to stem cell potential characteristics were completely inhibited. In addition, (D) it was confirmed that resistance to chemotherapy treatment was also overcome as the cell state was reversed by the regulated target. Credit: *Cancer Research* (2023). DOI: 10.1158/0008-5472.CAN-22-1559

This latest research result is the third in the development of reversal technology where lung cancer cells that had acquired metastatic traits returned to a state in which their metastatic ability was removed and drug sensitivity was enhanced.

More information: Namhee Kim et al, A cell fate reprogramming strategy reverses epithelial-to-mesenchymal transition of lung cancer cells while avoiding hybrid states, *Cancer Research* (2023). DOI: 10.1158/0008-5472.CAN-22-1559

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