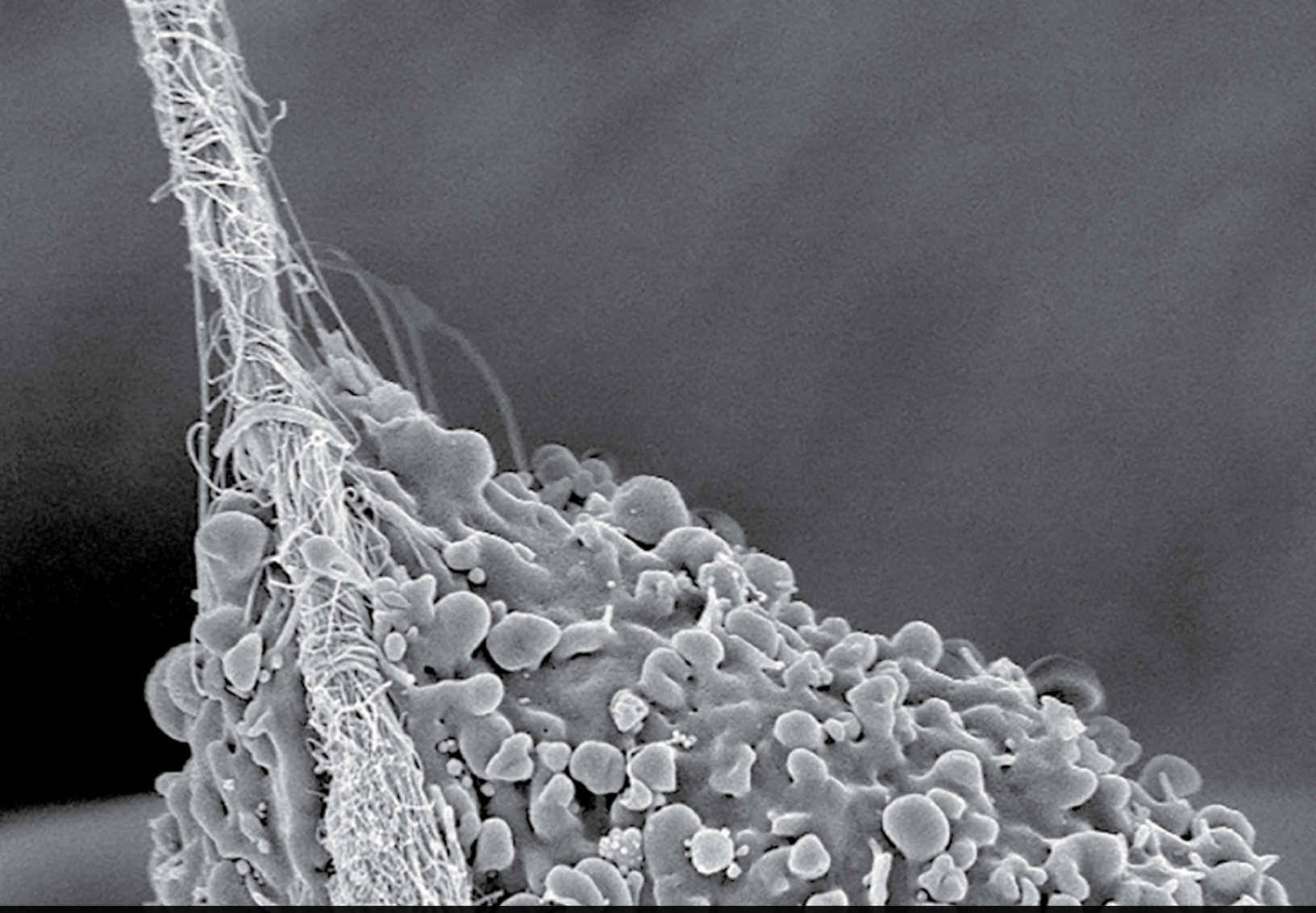

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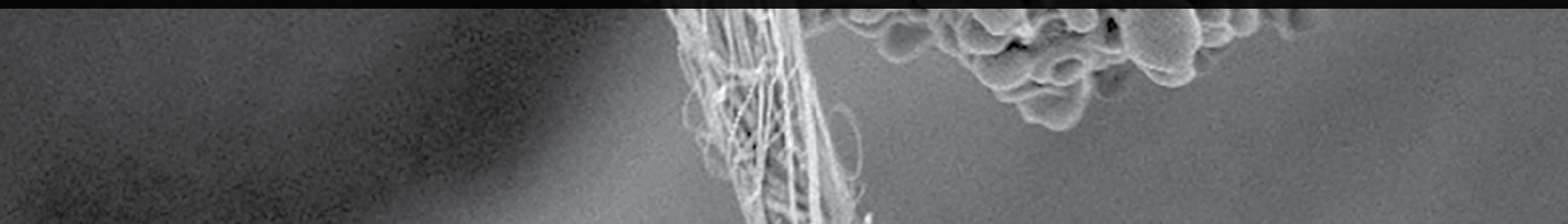

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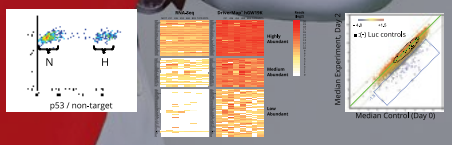

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JCB Journal of Cell Biology

CANCER CELL BIOLOGY 2020

The *Journal of Cell Biology* (*JCB*) publishes new cellular and molecular advances in any area of basic cell biology as well as studies that describe applied cell biology to translational fields like cancer biology. This collection celebrates works that advance our understanding of the fundamental molecular and cellular events shaping tumors and suggest novel potential therapeutic avenues, reflecting *JCB*'s scope and interest in cancer research, from core mechanistic findings to translational studies. Connect with *JCB* (jcellbiol@rockefeller.edu) with any feedback or questions, and visit www.jcb.org.

Brochure articles: Ben Short, PhD, Christina Szalinski, PhD, and Brittany Carson, PhD

Design: Christine Candia

On the cover: "The collagen trail: Journey to the microenvironment." Ovarian cancer cell migrating along a collagen fiber, imaged using scanning electron microscopy. Image © Elizabeth Harper, University of Notre Dame, Notre Dame, IN. This image was featured on the January 2020 cover of *JCB*.

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TUMOR SUPPRESSOR GENES SET THE DIVIDING LINE

The highly-conserved tumor suppressor genes Scribbled and Discs large work together with 14-3-3 proteins to control mitotic spindle positioning

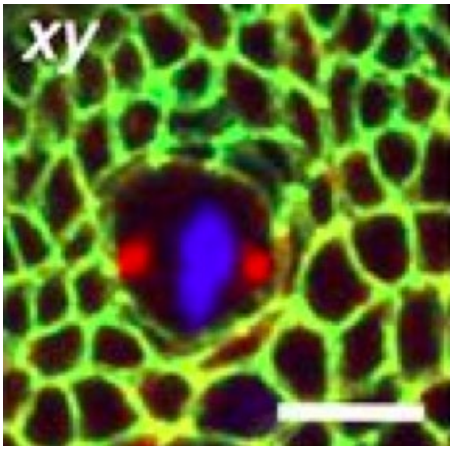
Tumor suppressor genes keep normal cells in check, but precisely how some of these genes function remains unknown. Matt Gibson, Investigator and Dean of the Graduate School at the Stowers Institute for Medical Research, together with first author and former postdoc Yu-ichiro Nakajima, now an Assistant Professor at Tohoku University, used the fruit fly *Drosophila* to provide new insights into the mechanism of action of the highly conserved tumor suppressor proteins Scribbled (Scrib) and Discs large (Dlg).

The vast majority of cells in our bodies reside in epithelial layers—polarized sheets of cells that tightly stick together. During epithelial cell proliferation, each mitotic spindle (the microtubule structure that works to separate chromosomes into two daughter cells) typically aligns parallel to the epithelial plane to ensure that the two daughter cells properly integrate into the tissue. This process is known as planar cell division. If the spindle is not aligned properly when it comes time for a cell to divide, tumors can develop or metastasis can occur. Nevertheless, exactly how the cell controls this alignment and coordinates division plane with epithelial polarity is not well understood. Nakajima and colleagues turned to *Drosophila* to learn more about the mechanisms of planar division.

Scrib and Dlg, along with another protein named Mud, are known to be important for spindle orientation. To understand how mitotic spindle movements are controlled by these proteins in real time, Nakajima and colleagues used live imaging in developing *Drosophila* wing epithelia where either Mud, Scrib or Dlg were depleted. The knockdown of Scrib or Dlg in the developing wing disc caused random spindle movements, "suggesting that Scrib and Dlg control spindle rotation and restrict spindle positioning," Nakajima says.

During cell division in the wing disc epithelium, Mud accumulates at spindle poles and is also localized to the cell junctions, where both Scrib and Dlg accumulate. Though Scrib and Dlg are found together, it was not known whether they interact at the junction, so the team expressed mutated versions of Scrib with various domains missing. The authors found that Scrib missing all of its PDZ domains reduced Dlg localization to junctions. Demonstrating a role for Scrib-Dlg interaction in planar spindle orientation, cells expressing that particular Scrib mutant also had abnormal planar spindle orientation.

A proteomic analysis using Dlg as bait revealed 14-3-3 proteins as potential binding partners. Using various 14-3-3 loss-of-function mutants Nakajima and colleagues were able to show that 14-3-3 proteins are required for proper control of planar spindle alignment. Additionally, knockdown of 14-3-3s or Dlg resulted in the reduction of physical associations between Scrib and Mud. "14-3-3 proteins could function as a molecular link that connects the junction-associated proteins Scrib/Dlg and the mitotic apparatus," Nakajima says.



In the *Drosophila* wing disc epithelium, Scrib (green) is found along junctions, while Mud (red) localizes at spindle poles during mitosis. DNA is shown in blue.

Credit: Nakajima et al., 2019

Because altered expression of Scribbled (Scrib), Discs large (Dlg), and 14-3-3 is associated with epithelial tumors in humans, the next step is to confirm that these proteins work the same way in mammalian tissue.

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ORIGINAL PAPER

Nakajima, Y., Z.T. Lee, S.A. McKinney , S.K. Swanson, L. Florens, and M.C. Gibson. 2019. Junctional tumor suppressors interact with 14-3-3 proteins to control planar spindle alignment. *J. Cell Biol.* 218:1824–1838.
https://doi.org/10.1083/jcb.201803116

NOVEL PRO-INVASIVE ENDOSOME FEEDBACK LOOP IS AMPLIFIED IN MUTANT P53 CANCER CELLS

Researchers use new technique to follow fast recycling endosomes

Most cancer-associated deaths occur due to metastasis—the process through which tumor cells acquire invasive capacity and disseminate to distant and often vital organs. The development of novel cancer treatment requires a better understanding of the acquisition of cell invasiveness and of metastasis, with a more in-depth assessment of pro-metastatic cellular processes downstream of the well characterized genetic and epigenetic alterations.

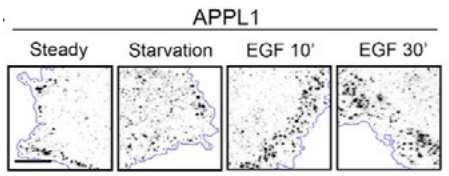
A team of researchers led by Sandra Schmid and Ping-Hung Chen at the University of Texas Southwestern Medical Center used a newly developed technique to monitor rapid endocytic trafficking, a process known to augment tumor progression and metastasis. This technique involves thick total internal reflection fluorescence (TIRF) microscopy and analysis using a specialized analysis platform, cmeAnalysis. The ability to follow fast recycling endosomes, previously a limiting factor in studies of endocytic trafficking, supports additional investigation into the role of early endosomes in normal and pathologic cell functions.

The team identified a subpopulation of endosomes at the cell edge that carry the signaling scaffold protein APPL1. These endosomes serve as hubs to modulate cell signaling and promote the rapid recycling of the cell adhesion molecule β1 integrin and the epithelial growth factor receptor (EGFR), thereby enhancing focal adhesion turnover and cell migration.

Although these APPL1-positive endosomes can be found in both normal and tumor cells, their selective redistribution to the cell perimeter is enhanced by GOF p53 through upregulation of both dynamin-1 (Dyn1) and myosin VI. This finding further elucidates the known link between GOF p53 mutations and endocytosis. Formation of these APPL1-positive endosomes depends on Akt signaling and, as increased APPL1 scaffold formation promotes Akt signaling and dynamin-1 activation, a positive feedback loop is created and can be further enhanced with GOF p53.

Endosomal recycling–receptor signaling crosstalk, in combination with feedback loops, can therefore be activated or amplified in cancer cells.

"In addition, our studies on the regulation of early endocytic trafficking in cancer cells have revealed added complexity with regard to the spatial organization and functional diversity of early endosomes," Schmid says.



Representative thick-TIRF immunofluorescence images of APPL1-positive endosomes with and without EGF signaling.
Credit: Lakoduk et al., 2019

The findings of this study have yet to be fully explored in vivo, but the relevance to patients and potentially patient care is supported by online patient databases that have confirmed the link between p53 mutations and increased *Dyn1* expression.

"Our data provide mechanistic insight into how selective activation of endocytic protein isoforms can alter endosomal recycling and receptor signaling to promote the adaptation required for aggressive phenotypes in cancers," Chen says.

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Lakoduk, A. M., P. Roudot, M. Mettlen, H. M. Grossman, S. L. Schmid, and P.-H. Chen. 2019. Mutant p53 amplifies a dynamin-1/APPL1 endosome feedback loop that regulates recycling and migration. *J. Cell. Biol.* 218:1928–1942.
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A DRUGGABLE TARGET FOR METASTATIC PROSTATE CANCER

Identification of the phosphatase PHLPP2's role in controlling MYC stability could also have implications for many other cancers

The oncogenic protein MYC drives cell growth and proliferation while enhancing cell metabolism and survival. It causes many different types of cancer but cannot be targeted by conventional drug therapies. "It is estimated that 450,000 Americans are diagnosed each year with a cancer that is driven by MYC," says Dawid G. Nowak, an assistant professor at Weill Cornell Medicine in New York.

One type of cancer associated with elevated MYC levels is metastatic prostate cancer. Around one in nine men will be diagnosed with prostate cancer during their lifetime. The disease is the second leading cause of cancer death among American men and is projected to kill over 30,000 people in 2019. The vast majority of these deaths are the result of cancers that spread, or metastasize, from the prostate to other organs in the body.

"The five-year survival of metastatic prostate cancer is only 28%, whereas the five-year survival of prostate-confined disease is almost 99%," explains Lloyd C. Trotman, a professor at Cold Spring Harbor Laboratory.

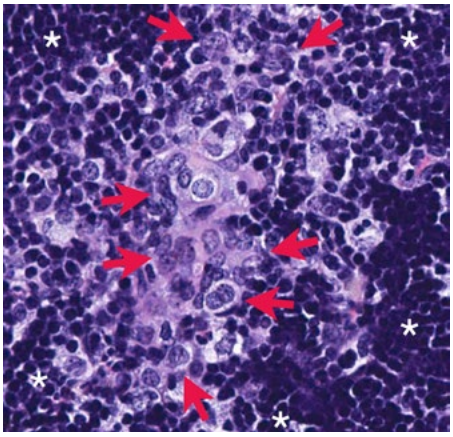
The phosphatase PHLPP2 is also elevated in metastatic prostate cancer cells, but the role of this protein was unclear. Nowak, Trotman, and colleagues found that metastatic prostate cancer cells require PHLPP2 to survive and proliferate. They discovered that PHLPP2 helps stabilize MYC by removing a phosphate group that would otherwise trigger MYC's destruction.

The researchers deleted the *Phlpp2* gene in mice and found that doing so prevented prostate cancer cells from metastasizing to other organs. This is significant because researchers have been unable to develop treatments that directly inhibit MYC, as it does not contain any features that can be easily targeted with a drug.

Trotman and colleagues then turned to human prostate cancer cells, which they treated with a drug that inhibits PHLPP2. This lowered MYC levels and caused the cells to stop proliferating and die.

PHLPP2 does not appear to perform any essential functions in healthy cells, so the researchers think that the enzyme could be an attractive way to indirectly target MYC in metastatic prostate cancer and possibly other cancers, too.

"Our results suggest that targeted efforts to design pharmacologically relevant PHLPP2 inhibitors could result in very efficient new drugs that suppress MYC-driven cancer," Trotman says.



Red arrows indicate prostate cancer cells that have metastasized to the lymph nodes of a genetically engineered mouse. This process is blocked in mice lacking the enzyme Phlpp2.

Credit: Nowak et al., 2019

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Nowak, D.G., K.C. Katsenelson, K.E. Watrud, M. Chen, G. Mathew, V.D. D'Andrea, M.F. Lee, M.M. Swamynathan, I. Casanova-Salas, M.C. Jibilian, C.L. Buckholtz, A.J. Ambrico, C.-H. Pan, J.E. Wilkinson, A.C. Newton, and L.C. Trotman. 2019. The PHLPP2 phosphatase is a druggable driver of prostate cancer progression. *J. Cell Biol.* 218:1943–1957.

https://doi.org/10.1083/jcb.201902048

CELL DEATH PATHWAY PROVIDES POSSIBLE ANTI-CANCER TARGETS

Modulating cell stress pathways that regulate levels of the pro-apoptotic protein BIK could help treat deadly triple-negative breast cancer

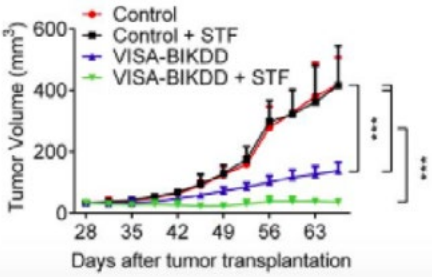
When a cell is stressed, a complex network of signals determine whether the cell will survive. Regulating these pathways can offer anti-cancer strategies. Fei-Yun Chen, Ruey-Hwa Chen, and colleagues at the Academia Sinica in Taiwan and National Taiwan University reveal a new understanding of what drives cell death under different stress conditions and how these pathways can be targeted to reduce tumor size in triple-negative breast cancer (TNBC) models.

Cell death regulation is crucial during development and for maintaining healthy tissue. When cell death is suppressed, cells can grow out of control, causing tumors to form. Regulating cell death pathways can influence cancer cells' sensitivity to anti-tumor treatments. A family of proteins known as Bcl-2 determine whether cells commit to death, and one of the proteins in the family is BIK. BIK is considered a pro-death protein; its presence signals a cell to die, but how BIK is regulated and its physiological functions were not well understood, nor was it known what stressors trigger BIK to promote cell death.

BIK protein has a short half-life and is thought to be flagged for destruction via a process known as ubiquitination. Chen and colleagues began searching for the protein involved in flagging it and identified Cul5-ASB11 as the ubiquitin ligase that modifies BIK and targets it for destruction. The researchers found that, in response to endoplasmic reticulum stress, ASB11 is transcriptionally activated by an effector of the stress-sensing protein IRE1α, promoting BIK degradation and cell survival. However, DNA damage–induced stress caused the tumor suppressor p53 to repress ASB11 through IRE1α, stabilizing BIK and promoting cell death.

"BIK ubiquitination and degradation are enhanced by ER stress and reduced by DNA damage, thereby oppositely regulating cell life/death decisions in the two stressed conditions," Ruey-Hwa Chen says. This presents "an intriguing crosstalk between different cellular stress pathways."

In TNBC, a highly aggressive disease with limited treatment options, the anti-tumor strategy of expressing an active BIK mutant is ineffective because the mutant is prone to degradation, which helps keep TNBC cells alive. In both cell lines and a mouse model of TNBC, overexpressing the active BIK mutant in combination with an IRE1α inhibitor showed enhanced anti-tumor effects compared to either treatment by itself.



In a mouse model of triple-negative breast cancer, overexpressing an active BIK mutant (VISA-BIKDD) in combination with an IRE1α inhibitor (STF) showed enhanced anti-tumor effects compared to either treatment by itself.

Credit: Chen et al., 2019

"Targeting the BIK degradation pathway in combination with the administration of an active BIK mutant could offer an effective anti-cancer strategy," Chen says.

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ORIGINAL PAPER

Chen, F.Y., M.Y. Huang, Y.M. Lin , C.H. Ho , S.Y. Lin , H.Y. Chen , M.C. Hung, and R.H. Chen. 2019. BIK ubiquitination by the E3 ligase Cul5-ASB11 determines cell fate during cellular stress. *J. Cell Biol.* 218:3002–3018.
https://doi.org/10.1083/jcb.201901156

SRC TARGETS THE TUMOR SUPPRESSOR DLC1

Interaction between DLC1 and tumor promoting kinase SRC reveals a possible new treatment route for DLC1-positive tumors

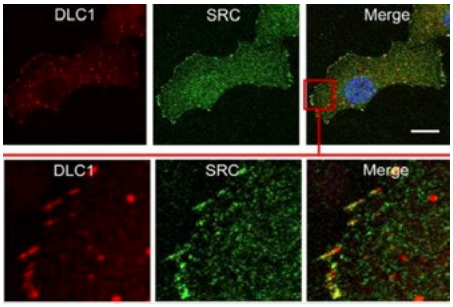
Personalized treatments hold promise for cancers that evade generic chemotherapies. Research by Brajendra Tripathi, Douglas Lowy, and colleagues at the National Cancer Institute indicates that it may be possible to target a subset of cancers that express the tumor suppressor and focal adhesion protein DLC1, a component of focal adhesions that can switch off the active GTPase RhoA by acting as a GTPase-activating protein (GAP). They establish, for the first time, a connection between DLC1 and the tumorigenesis-promoting kinase SRC, revealing SRC inhibition can be an effective part of the tumor treatment in a DLC1-positive cancer model and that reactivation of the tumor suppressor can be a potent anti-tumor approach.

SRC is a kinase that controls many tumor-promoting processes, including regulating the cytoskeleton and a cell's ability to move and attach, by changing the expression of oncogenic and tumor suppressor genes. SRC also regulates the RhoA GTPase, a protein that is frequently activated in advanced cancer. While surveying protein expression in cancer-derived cell lines, Tripathi, Lowy, and colleagues found a strong and unexpected correlation between the levels of RhoA-GTP, total SRC protein, and SRC activity and an inverse correlation with DLC1 protein levels.

To explore a possible mechanistic relationship between SRC, RhoA-GTP, and DLC1, they treated two DLC1-positive and two DLC1-negative non–small cell lung cancer lines with the SRC inhibitor Saracatinib, which reduced RhoA-GTP in both DLC1-positive lines, but not in the DLC1-negative lines. These results indicate SRC kinase can increase RhoA-GTP in DLC1-positive cells, and the SRC inhibitor can reverse the process.

Using co-immunoprecipitation assays, they found that SRC and DLC1 interact and that the localization of SRC to focal adhesions depends on the presence of DLC1 protein. Their results show that SRC binds directly to DLC1 and phosphorylates it at residues Y451 and Y701. Phosphorylation analysis indicated that DLC1 can also be phosphorylated on S129 by the kinase ERK, which increases both the binding of SRC to DLC1 and SRC-dependent phosphorylation of DLC1. Phosphorylation of DLC1 by SRC attenuates DLC1's Rho-GAP activity, the researchers discovered. Cells with DLC1 mutations that are deficient for SRC phosphorylation lacked well-formed stress fibers, like those found in cells with an active DLC1.

"Given the reversibility of the SRC-dependent DLC1 phosphorylation, we evaluated whether SRC inhibitors might have therapeutic efficacy in a DLC1-positive tumor that had high SRC activity," Tripathi, Lowy, and colleagues said. A SRC inhibitor by itself reduced the size of DLC1-expressing tumors in mice by 64%, and combining it with an inhibitor of AKT, which also phosphorylates and attenuates the tumor suppressor activity of DLC1, reduced tumor size by 89%.



Colocalization between DLC1 (red) and SRC (green) in a DLC1-positive non–small cell lung cancer cell line.
Credit: Tripathi et al., 2019

"One possible way to increase the proportion of tumors for which the therapeutic targeting of DLC1 could be clinically beneficial might be to use a suitable inhibitor to reverse an epigenetic change that has resulted in reduced or silenced DLC1 expression... and to combine this treatment with inhibition of SRC and/or AKT kinase activities," Tripathi, Lowy, and colleagues say.

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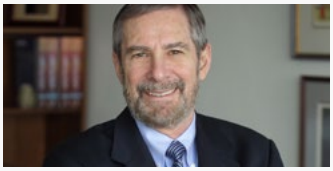

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ORIGINAL PAPER

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https://doi.org/10.1083/jcb.201810098

HAIR FOLLICLES RESTRICT CANCER GROWTH

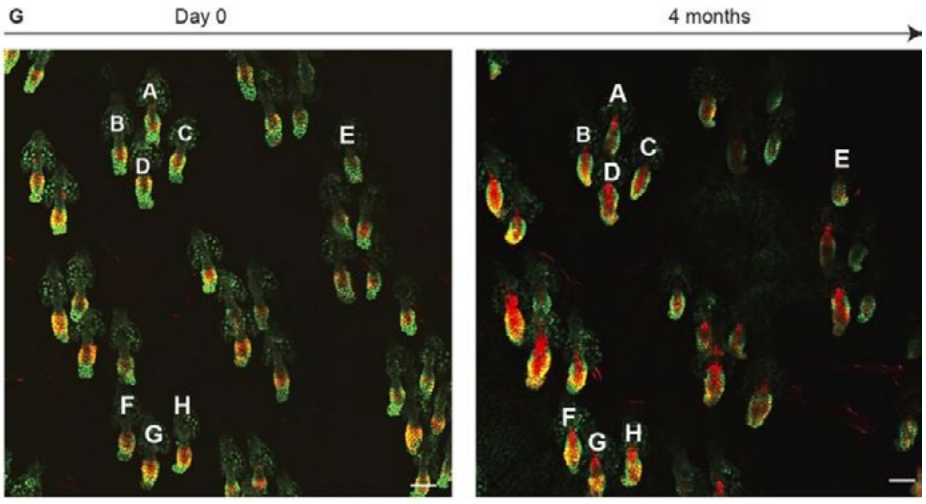
Normal skin corrals cancer-causing mutations and reveals how tissue can subvert tumorigenesis

Normal aged skin contains cancer-causing mutations, recent research has shown, but how these mutations are prevented from forming tumors was a mystery. Christiana M. Pineda, Valentina Greco, and colleagues at Yale University used unique live-tissue imaging to track mouse skin cells after inducing cancer-causing mutations. They found that protection against skin cancer comes from a surprising place: hair follicles.

About 30% of all cancers contain a Ras mutation, but these same mutations have been found in non-cancerous skin epithelia. Pineda and colleagues induced Ras mutations in mouse hair follicle cells along with a glowing red reporter to track the mutant stem cells and their progeny. The cells persisted in the epithelium, revealing that the body doesn't just eliminate mutant cells.

Pineda and colleagues found that when they induced mutations in hair follicle stem cells, they outcompeted wild-type neighboring cells. They still responded to normal tissue constraints, such as resting phase cues, however. Even after a year, the transformed cells did not develop into tumors. In contrast, targeting the Ras mutation to the upper non-cycling region of the skin epithelium led to benign outgrowths.

Introducing a second mutation that results in the loss of TGFβ signaling into Ras-mutant hair follicle stem cells did induce some tumors, typically at sites of high grooming or scratching. Imaging revealed that those tumors arose after an injury caused them to exit the follicular niche. To test whether injury can promote tumorigenesis within the follicle, they ablated hair follicle bulbs and the double mutant cells showed rapid, and normal, regeneration. "Once out of the follicular niche, Hras mutant cells can no longer be controlled and contained through hair regeneration programs," Pineda says.



Two-photon images of the ear skin from the same mouse at day 0 and 4 months after Ras activation reveal normal follicular architecture despite the persistent presence of mutant cells (red).
Credit: Pineda et al., 2019

"Our results indicate that the hair follicle has a unique ability to cope with Ras-activated cells. This organ is able to integrate the mutant epithelial cells while remaining clinically normal," Pineda says. There's still much to learn about what's going on in the skin that could be applied to other cancers. "Manipulation of certain cell types or signaling pathways may enable and/or enhance the ability of other epithelial tissues to also suppress oncogenic growth."

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https://doi.org/10.1083/jcb.201907178

PROTEIN INVOLVED IN CANCER METASTASIS HIJACKS ANOTHER FOR TRANSPORT

A critical protease for metastasis uses a protein involved in intracellular transport to get to the cell surface

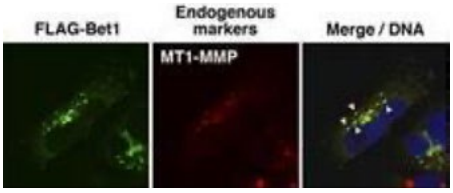
Metastasis is often a death sentence for cancer patients. When a cancer cell begins to invade surrounding tissue, it forms a protrusion called an invadopodium. Matrix metalloproteinases (MMPs) are critical regulators of this process. Takuya Miyagawa, Kana Hasegawa, Yoko Aoki, Takuya Watanabe, Hiroki Inoue, and colleagues at Tokyo University of Pharmacy and Life Sciences reveal a novel mechanism for how one of those MMPs, membrane type 1–MMP (MT1-MMP), is delivered to invadopodia.

Many MMPs involved in cancer cell invasion are secreted, soluble enzymes, but MT1-MMP is membrane bound. MT1-MMP is synthesized in the endoplasmic reticulum (ER) and transported in vesicles to invadopodia, which are structures with the ability to degrade the extracellular matrix, but the molecular mechanism underlying this intracellular transport was not fully understood.

One crucial family of proteins involved in vesicle trafficking specificity are SNAREs, which act like twist ties when they meet on opposing membranes, driving the fusion of a vesicle with its destination membrane. "Each SNARE is localized in a unique organelle and forms specific complexes with cognate SNAREs to ensure membrane fusion specificity," Miyagawa says. The team identified Bet1 as the SNARE that is required for extracellular matrix degradation. Bet1 was previously shown to be involved in transport from the ER to the Golgi apparatus.

They found Bet1 colocalized with the Golgi, but surprisingly also with MT1-MMP in late endosomes. In invasive cells, the team found that Bet1 was able to reach the cell membrane, but in non-invasive cells, it stayed in the Golgi. Bet1 appears to be involved in MT1-MMP delivery to the cell surface, as Bet1 knockdown decreased the amount of MT1-MMP that reached the membrane, whereas Bet1 overexpression increased MT1-MMP delivery. Additionally, in invasive cells, Bet1-GFP was found in areas associated with invadopodia maturation.

Together, the team's data indicates that MT1-MMP diverts Bet1 from its function in ER to Golgi transport, to promote MT1-MMP trafficking to the cell surface, including to invadopodia in invasive breast cancer cells. "MT1-MMP hijacks



Bet1 (green) colocalizes with MT1-MMP (red) in invasive breast cancer cells.
Credit: Miyagawa et al., 2019

Bet1 function for its own transport," Miyagawa says. Also, the team showed that in invasive cells Bet1 is localized in MT1-MMP–positive endosomes as well as the Golgi apparatus, and it forms a novel SNARE complex with syntaxin 4 and endosomal SNAREs.

"MT1-MMP changes the function of Bet1 for its efficient transport to invadopodia. Bet1 is critical for the formation of functional invadopodia that degrade ECM, and therefore could be a novel target for diagnosis, treatment, and prognosis prediction of the disease," Miyagawa says.

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CANCER CELLS TURN TO CANNIBALISM TO SURVIVE CHEMOTHERAPY

Senescent cancer cells can engulf and digest their neighbors, allowing them to stay alive and initiate tumor relapse

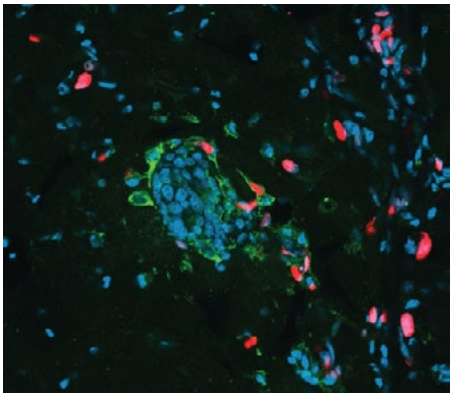
Chemotherapy drugs like doxorubicin kill cancer cells by damaging their DNA, but cells that survive initial treatment can soon give rise to new tumors. This is a particular problem in breast cancers that retain a normal copy of the *TP53* gene. Instead of dying in response to chemotherapy-induced DNA damage, these cancer cells generally just stop proliferating and enter a dormant but metabolically active state known as senescence. In addition to surviving chemotherapy, these senescent cancer cells produce large amounts of inflammatory molecules and other factors that can promote tumor regrowth. Chemotherapy-treated breast cancer patients with normal *TP53* genes are therefore prone to relapse and have poor survival rates.

"Understanding the properties of these senescent cancer cells that allow their survival after chemotherapy treatment is extremely important," says Crystal A. Tonnessen-Murray, a postdoctoral research fellow in James G. Jackson's laboratory at the Tulane University School of Medicine.

Tonnessen-Murray and colleagues found that, after exposure to doxorubicin or other chemotherapy agents, breast cancer cells that become senescent often engulf neighboring cancer cells. The researchers observed this surprising behavior not only in cancer cells in vitro, but also in orthotopic tumors growing in mice. Lung and bone cancer cells are also capable of engulfing their neighbors after becoming senescent, the researchers discovered.

Tonnessen-Murray and colleagues found that senescent cancer cells upregulate a group of genes that are involved in phagocytosis and are normally active in macrophages. After "eating" their neighbors, senescent cancer cells digest them by delivering them to lysosomes, degradative organelles that are also highly active in senescent cells.

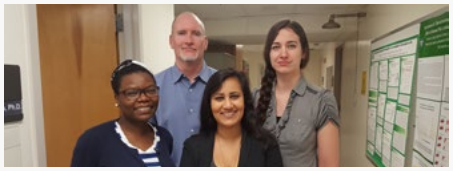
Importantly, the researchers determined that this process helps senescent cancer cells stay alive. Senescent cancer cells that engulfed a neighboring cell survived in culture for longer than senescent cancer cells that didn't. The researchers suspect that consuming their neighbors may provide senescent cancer cells with the energy and materials they need to survive and produce the factors that drive tumor relapse.



A breast tumor formed in mice and treated with doxorubicin contains some cancer cells (red nuclei) that have been engulfed by other cancer cells (green cell membrane).
Credit: Tonnessen-Murray et al., 2019

"Inhibiting this process may provide new therapeutic opportunities, because we know that it is the breast cancer patients with tumors that undergo *TP53*-mediated senescence in response to chemotherapy that have poor response and poor survival rates," Jackson says.

RESEARCHER DETAILS



Jackson (back left) and Tonnessen-Murray (back right) pictured with co-authors Joy Olayiwola (front left) and Sonia Rao (front right).

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Tonnessen-Murray, C.A., W.D. Frey, S.G. Rao, A. Shahbandi, N.A. Ungerleider, J.O. Olayiwola, L.B. Murray, B.T. Vinson, D.B. Chrisey, C.J. Lord, and J.G. Jackson. 2019. Chemotherapy-induced senescent cancer cells engulf other cells to enhance their survival. *J. Cell Biol.* 218:3827–3844.
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IMPORTIN-11 MEDIATES NUCLEAR IMPORT OF βCATENIN

Targeting this transport step may block the growth of colorectal cancers caused by mutations in *APC*

Around 80% of colorectal cancers are associated with mutations in the *APC* gene that stabilize the transcription factor βcatenin and lead to the protein's accumulation in the cell nucleus, where it can activate numerous genes that drive cell proliferation and promote the growth and maintenance of colorectal tumors. But how βcatenin enters the cell nucleus after its levels rise is poorly understood.

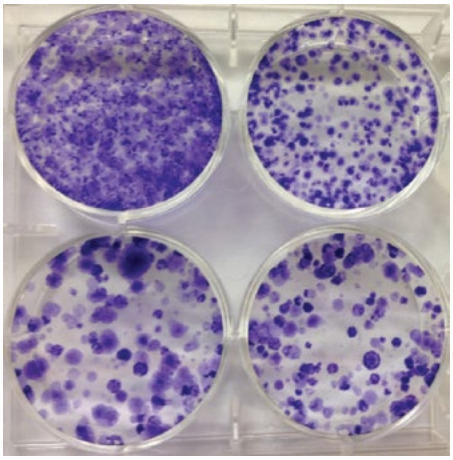
"Because the molecular mechanisms underlying βcatenin nuclear transport remain unclear, we set out to identify genes required for continuous βcatenin activity in colorectal cancer cells harboring *APC* mutations," says Stephane Angers, a professor in the Department of Pharmaceutical Sciences at the University of Toronto's Leslie Dan Faculty of Pharmacy.

Angers and colleagues, including first author Monika Mis, developed a new technique that allowed them to screen the human genome for genes that support βcatenin's activity in *APC*-mutant colorectal cancer cells. The DEADPOOL technique uses a colon cancer cell line engineered to express an inducible form of the apoptosis-inducing protease Caspase-9 in a βcatenin-dependent manner. Due to their high βcatenin levels, most cells die in response to Caspase-9 induction. But cells will survive if they lack genes required to maintain βcatenin transcriptional activity. "The DEADPOOL platform constitutes a robust system to conduct genetic suppressor screens for the identification of genes involved in signaling systems," Mis says.

One of the top hits in a CRISPR-based screen of the DEADPOOL cells was *IPO11*, which encodes a protein called Importin-11 that is known to be involved in nuclear import. Angers and colleagues found that Importin-11 binds to βcatenin and escorts it into the nucleus of colorectal cancer cells with mutations in *APC*. Removing Importin-11 from these cells prevented βcatenin from entering the nucleus and activating its target genes.

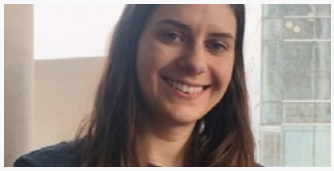
The researchers discovered that Importin-11 levels are often elevated in human colorectal cancers. Moreover, removing Importin-11 inhibited the growth of tumor organoids formed by *APC* mutant cancer cells isolated from patients.

"We conclude that Importin-11 is required for the growth of colorectal cancer cells," Angers says. Learning more about how Importin-11 transports βcatenin into the nucleus may help researchers develop new therapies that block this process and reduce the growth of colorectal cancers caused by mutations in *APC*.



Compared with a control (top left), removal of βcatenin (top right) or Importin-11 (bottom left and bottom right) reduces the growth of colorectal cancer cells carrying a mutation in *APC*.
Credit: Mis et al., 2019

RESEARCHER DETAILS



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ORIGINAL PAPER

Mis, M., S. O'Brien, Z. Steinhart, S. Lin, T. Hart, J. Moffat, and S. Angers. 2020. IPO11 mediates βcatenin nuclear import in a subset of colorectal cancers. *J. Cell Biol.* 219:27–39.
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CHROMOSOMES ARE A BARRIER TO NORMAL CELL DIVISION IN POLYPLOID CELLS

Duplicated chromosome sets is common in cancer but the extra chromosomes act as a barrier to typical "bipolar" cell division

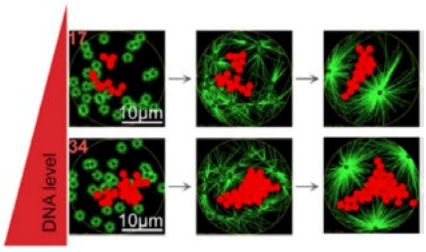
Polyploidy, when an entire duplicated chromosome set is maintained within a cell, is common in human tumors. How cells maintain this unwieldy extra DNA, especially when it comes time to divide, was not well understood. Alix Goupil, Maddalena Nano, Renata Basto, and colleagues at the Institut Curie in Paris, France studied how polyploid cells divide and found that the chromosomes act as a barrier to typical "bipolar" cell division, but microtubule stability promotes bipolar cell division.

Though polyploidy is associated with poor prognosis in cancer, it is also a programmed and regulated process that normally occurs in early mammalian development. Paradoxically, cells that acquire an extra set of chromosomes through normal regulation have a hard time proliferating, but tumors can form when polyploid cells arise from errors in cell division. In a normal "bipolar" arrangement before cell division, two centrosomes line up and serve as microtubule-organizing centers at either side of the cell. The microtubules then attach to the chromosome's kinetochores, pull the chromosomes apart, and the cell splits in two, in a process known as cytokinesis. Cells with more than two centrosomes can still form a bipolar spindle by forming centrosome clusters at each side of the cell. But things get even more complicated when there are extra chromosomes as well as extra centrosomes.

Goupil and colleagues focused on cells with polyploidy and extra centrosomes due to errors in cytokinesis. They induced cytokinesis failure in *Drosophila* neural stem cells and found that the extra centrosomes clustered in more than two groups, while the chromosomes adopted a multilobed arrangement within a multipolar spindle. Most polyploid anaphases were multipolar and generated several nuclei at mitotic exit. The extra chromosomes of polyploid cells seemed to prevent spindle bipolarity.

Similarly, in an in silico program designed to simulate cell division with various numbers and arrangements of centrosomes and DNA, bipolar spindle assembly was inhibited by the presence of extra DNA. In an ovarian cancer cell line with polyploidy induced by cytokinetic failure, the researchers confirmed that chromosomes acted as a barrier to spindle pole coalescence.

Because their simulations also showed that stabilizing microtubules improved centrosome clustering and the formation of a bipolar spindle, the researchers looked at ovarian cancer cells lacking the microtubule-depolymerizing kinesin MCAK. "This microtubule stabilization resulted in a considerable improvement in spindle bipolarity, and a large majority of polyploid cells divided in a bipolar manner," says Goupil. "In light of our findings and knowing that whole genome duplications are frequent in cancer, it is possible that clonal expansion of polyploid cancer cells is favored in conditions of increased microtubule stability."



Computer simulations with variable amounts of DNA (red) show that increase in DNA promote multipolar spindle arrangements.
Credit: Goupil et al., 2020

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Goupil, A., M. Nano, G. Letort, S. Gemble, F. Edwards, O. Goundiam, D. Gogendeau, C. Pennetier, and R. Basto. 2020. Chromosomes function as a barrier to mitotic spindle bipolarity in polyploid cells. *J. Cell Biol.* 219:1824–1838.
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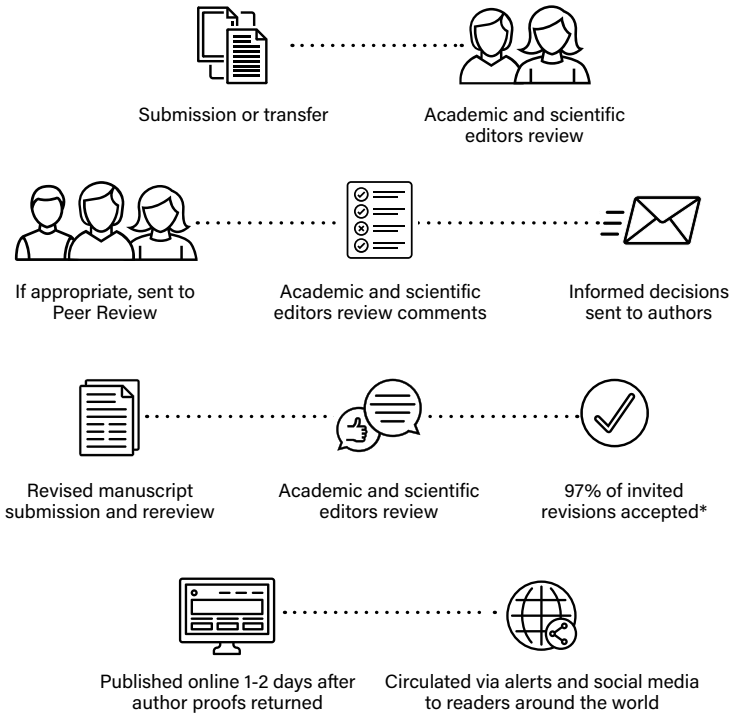

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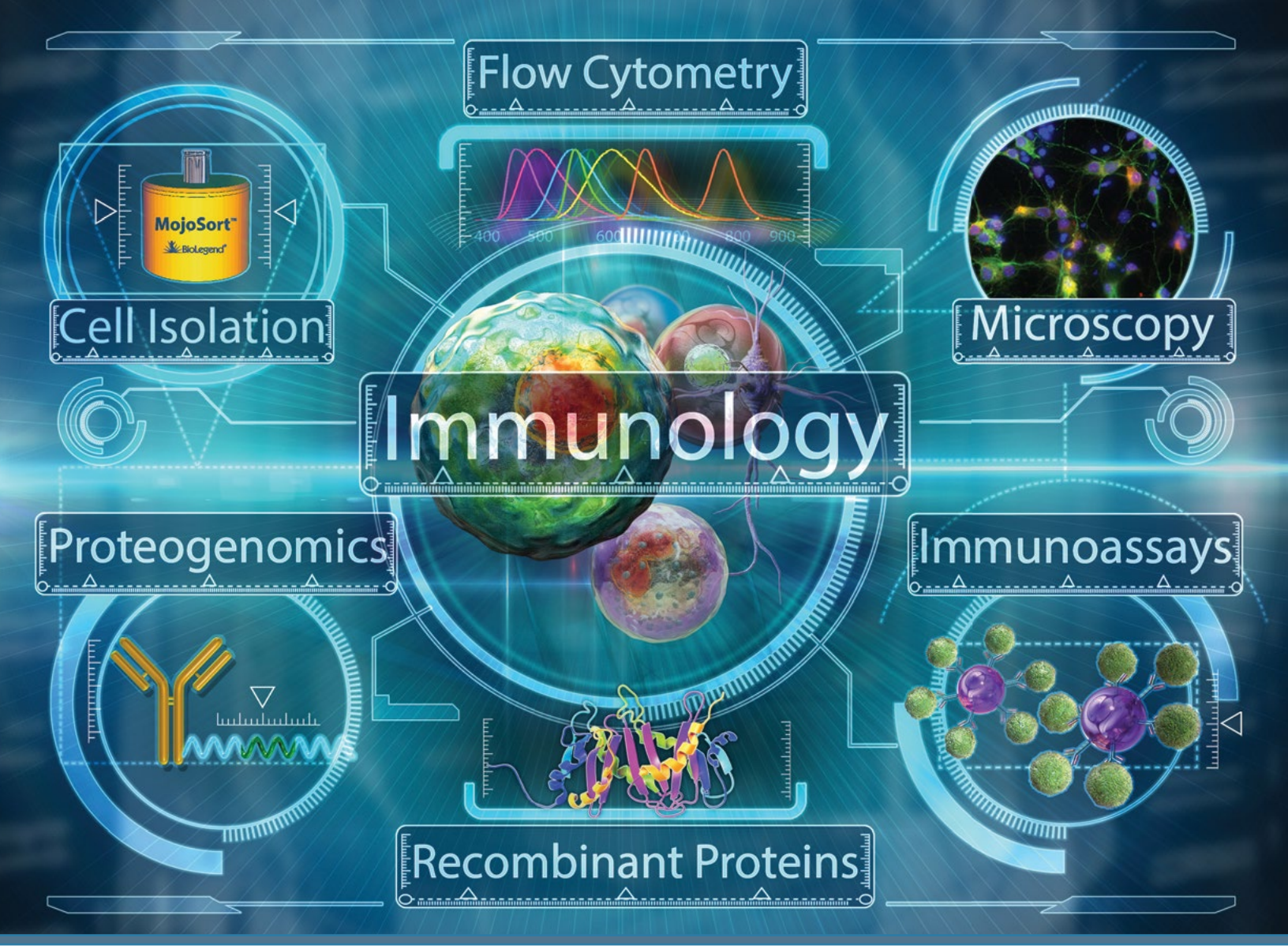

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