Discovery of novel cancer signaling mechanism and design of new anticancer compound

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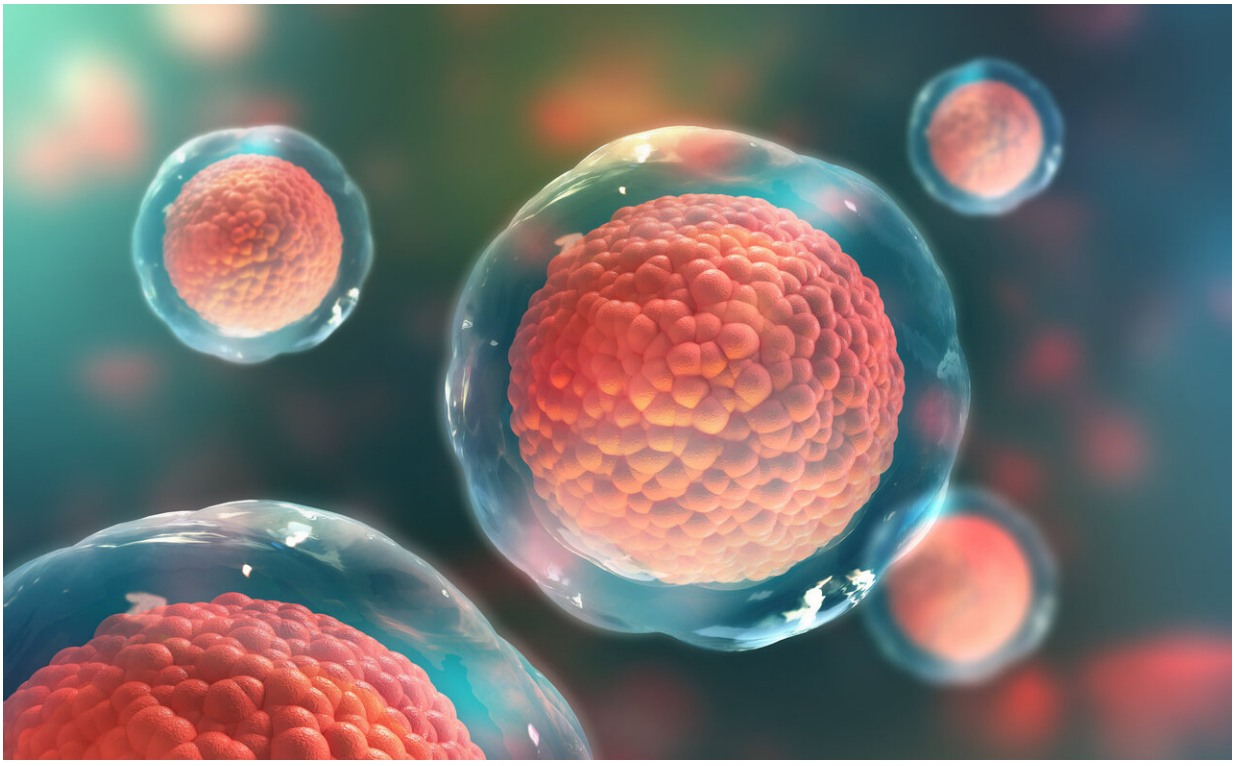

Scientists uncover novel signaling mechanism in cancer and also propose new channel of treatment Credit: © Tokyo University of Science

Active mutations of a certain signaling receptor protein called KIT tyrosine kinase are found in several cancers, such as acute myeloid

leukemia (AML). However, the different locations in the AML cells where KIT induces cancer-specific signaling remain unclear. Now, a group of scientists from Japan has aimed to answer this question by using a newly synthesized compound (along with other existing ones) that targets intracellular transport, which may offer an attractive strategy to combat cancer.

Of the various different functions that proteins perform in a cell, a crucial one is the recognition and transmission of certain "signals," collectively referred to as signal transduction. Receptors (proteins) on the cell surface recognize certain molecules and then initiate a chain of biochemical events inside the cell. These biochemical events are responsible for cellular activities such as multiplication, survival, etc. Needless to say, any perturbation of this "biochemical signaling" can be extremely detrimental to the cell, even leading to cancer in some cases. But, it is possible to target defective biochemical signaling pathways within a cell to treat cancer, provided the underlying mechanisms are studied thoroughly. This is exactly what a group of scientists from Japan set out to do in their study published in *Cell Communication and Signaling*. This research group—whose study was supported by Japan Agency for Medical Research and Development (AMED)—was headed by Assoc. Prof. Yuuki Obata from Tokyo University of Science (also National Cancer Center), and consists of Prof. Isamu Shiina (Tokyo University of Science), Prof. Ryo Abe (Tokyo University of Science and Teikyo University), Dr. Toshirou Nishida (National Cancer Center), and Prof. Koji Okamoto (National Cancer Center).

A certain signaling receptor protein called KIT tyrosine kinase functions in the growth and survival of different types of cells, including hematopoietic cells (the progenitors of all blood cells), mast cells (a type of immune cells), and interstitial cells of Cajal (electrical pacemakers in gastrointestinal tract). Active mutations of this protein have been identified in several cancers, such as mast cell leukemia (MCL),

gastrointestinal stromal tumor (GIST), and acute myeloid leukemia (AML). In MCL, the mutations *D816V* (human) and *D814Y* (mouse) are frequently found; here, the mutated KIT protein "mislocalizes" in a cellular compartment called the "endolysosome" (EL). In GIST, mutated KIT accumulates in and conducts cancer-specific signaling from the Golgi, the site in a cell where macromolecules are produced, modified, and packaged, especially proteins. Active KIT mutations have been found in about 10% of core binding factor AML (CBF-AML) patients; these are also associated with poor prognosis in AML. However, it remains unclear whether KIT transduces signals from intracellular compartments in AML. The research group from Japan aimed to answer this question by using a newly synthesized compound called M-COPA (along with other existing ones) that targets intracellular transport. According to them, this also represents an attractive strategy to combat cancer. Prof Shiina candidly says, "We wanted to investigate the anticancer effect of the new anticancer drug lead compound M-COPA synthesized at our university against hematological cancers (leukemia, lymphoma, etc.)."

Apart from *D816V*, another major active KIT mutation in AML is *N822K*. *D816V* has been characterized extensively, but the signaling platforms and mechanisms of *N822K* are relatively unknown. Also, before this study, it was unclear how the mutated KIT and where the downstream signaling molecules are activated. The scientists investigated the relationship between the localization of KIT^{N822K} (KIT protein carrying the *N822K* mutation) and its activation in an AML cell line, Kasumi-1. The scientists found that in AML cells, KIT^{N822K} mislocalized to and accumulated in the EL. Newly produced KIT in the endoplasmic reticulum (ER) travels to the cell membrane via the Golgi and then relocates to EL. However, immunofluorescence experiments (those that use antibodies against the mutated KIT, tagged with fluorescent dyes for identification) showed that KIT was activated in the Golgi. KIT activation on the Golgi was also found in other leukemia cells that have

the receptor mutation.

Next, Prof. Shiina and colleagues found that in the Golgi in AML cells, KIT^{N822K} also activates downstream signaling proteins called AKT, ERK, and STAT5. They did this by using specific compounds that target intracellular transport of proteins: brefeldin A (BFA), 2-methylcoprophilinamide (M-COPA) (inhibitors of transport from ER to the Golgi), and monensin (inhibitor of Golgi export). They found that in cells treated with BFA or M-COPA, KIT was retained in the ER. This also decreased the auto-phosphorylation of KIT and thereby its downstream signaling. Suppression of Golgi export of KIT using monensin did not suppress the KIT signals, which told the scientists that mutated KIT carries out cancer signaling specifically at the Golgi.

So, what are the future applications of this study? Small molecule TKIs (tyrosine kinase inhibitors) and antibodies against RTKs (receptor tyrosine kinases) have been developed to suppress cancer proliferative signaling using mechanisms similar to the ones described above. According to Prof. Shiina and the group, this study reveals that the novel compound M-COPA can be used to block transport of KIT from the ER to the Golgi (where it is activated and carries out downstream oncogenic signaling). The scientists say that the compound M-COPA has applications such as treatment of patients with AML, improved prognosis for these patients, and improvement in the quality of life of these patients. Prof. Shiina concludes by stating, "Currently, the synthesis of various M-COPA analogs is progressing every day at our university, and their inhibitory effects against hematological cancers and solid cancers (stomach cancer, lung cancer, ovarian cancer, etc.) are being verified."

More information: Yuuki Obata et al, N822K- or V560G-mutated KIT activation preferentially occurs in lipid rafts of the Golgi apparatus in leukemia cells, *Cell Communication and Signaling* (2019). DOI:

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