Researchers identify how 'phenotype switching' can make melanoma become metastatic and resistant to drugs

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(Medical Xpress)—One of the challenges of understanding cancer is trying to determine the mechanisms that drive metastasis, or the process by which tumor cells are able to spread throughout the body. In order to investigate metastasis, researchers at The Wistar Institute focused on a process involving the phenotypes – the outward, physical appearance based on genetic coding – of tumor cells. According to the researchers, "phenotype switching" may be involved in changing appearance of melanoma tumors by altering the number and type of protein receptors that dot the surface of the individual melanoma cells within the tumor. Identifying the phenotype patients exhibit may help determine which patients are more likely to benefit from existing medications while also providing an opportunity to create new targeted therapies.

The findings were published in the journal *Cancer Discovery* and are currently available online.

"We were able to demonstrate for the first time that different receptors within a single signaling pathway – in this case, the Wnt signaling pathway – can guide the phenotypic plasticity of tumor cells, and increased signaling of Wnt5A in particular can result in an increase in highly invasive tumor cells that are less sensitive to existing treatments for metastatic melanoma," said Ashani Weeraratna, Ph.D., assistant professor in the Tumor Microenvironment and Metastasis Program of Wistar's NCI-designated Cancer Center, and senior corresponding author

on the manuscript.

While melanoma accounts for less than 5 percent of all cases of skin cancer, it is the deadliest form of the disease, resulting in a large majority of all the deaths related to skin cancer, according to the American Cancer Society. The five-year survival rate for patients with metastatic melanoma is between 15 and 20 percent, and while new, targeted therapies designed to combat the disease based on a person's genetics have become available in recent years, some of these drugs are not particularly effective in many patients, and many who do respond well to the drugs often eventually become resistant to them. This makes understanding advanced stages of melanoma and what internal processes could be at work all the more important for developing new treatments.

Weeraratna and her team focus on Wnt5A, a Wnt signaling molecule that has been found in increased levels in metastatic melanomas. In order for Wnt5A to promote the phenotype switch from early in the tumor's formation to the time it becomes metastatic, the tyrosine kinase receptor ROR2 is required. When ROR2 is not present, Wnt5A is unable to promote tumor metastasis. The only other member of the family that has been identified is ROR1, and this research was done to determine what role ROR1 might play in the progression of melanoma.

The researchers were able to determine that ROR1 inhibited the invasion of melanoma cells, and this receptor was targeted for degradation by Wnt5A and ROR2. When ROR1 was silenced, the researchers observed that there was an increased rate of invasion of melanoma cells both in vitro and in vivo. The researchers also found that hypoxia – areas of low oxygen supply in the tumor – is able to induce a switch from ROR1 to ROR2 and results in an increase in levels of Wnt5A, suggesting the switch from a non-invasive ROR1-positive phenotype to an invasive ROR2-positive phenotype occurs when the tumor is exposed to hypoxic conditions. The researchers also found that a protein HIF1α is required

to increase the Wnt5A expressed. When HIF1α was removed, ROR2 was decreased, indicating that the upregulation of ROR2 via HIF1α requires Wnt5A.

To determine the clinical implications, the researchers hypothesized that melanoma cells driven by the mutant BRAF gene that were also high in Wnt5A might be less sensitive to treatment with vemurafenib, a drug approved by the FDA in 2011 for the treatment of BRAF-positive metastatic melanoma. By measuring BRAF cell lines to determine their Wnt5A and ROR2 status, as well as their sensitivity to BRAF inhibitors, the researchers found a significant correlation between BRAF inhibitor resistance and Wnt5A expression. Additionally, in a small cohort of patients, they found that seven out of nine patients who demonstrated less than a 33 percent clinical response to vemurafenib had a positive expression of Wnt5A, and only two of the remaining 15 patients who had a 38% or greater clinical response to vemurafenib exhibited any Wnt5A expression. When they silenced ROR2 in tumors xenografted in mice, the tumors responded far better to simultaneous treatment with the BRAF inhibitor vemurafenib. Conversely, when ROR1 was silenced, the tumors became more resistant to the drug.

Additionally, in eight patients who had undergone BRAF inhibitor therapy, the levels of Wnt5A were much lower in tumor cells prior to therapy compared to cells that were tested for Wnt5A after those same patients had relapsed.

"By using Wnt5A as a biomarker, we could determine which patients are likely to respond better to therapy with vemurafenib and help prolong that response," Weeraratna said. "There is also the potential to explore small molecule inhibitors of ROR2, since there is now a clear association between that and the ability of melanoma to become not only metastatic, but also therapy-resistant. This link between metastasis and therapy resistance is what we find the most exciting and intriguing, as therapies

designed to target one process may have a significant impact on the other as well. "

More information: cancerdiscovery.aacrjournals.o … .CD-13-0005.abstract

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