MedicalXpress

Circulating tumor cell clusters more likely to cause metastasis than single cells

August 28 2014

Circulating tumor cell (CTC) clusters – clumps of from 2 to 50 tumor cells that break off a primary tumor and are carried through the bloodstream – appear to be much more likely to cause metastasis than are single CTCs, according to a study from investigators at the Massachusetts General Hospital (MGH) Cancer Center. Their report in the August 28 issue of *Cell* also suggests that a cell adhesion protein binding CTC clusters together is a potential therapeutic target.

"While CTCs are considered to be precursors of metastasis, the significance of CTC clusters, which are readily detected using devices developed here at MGH, has remained elusive," says Shyamala Maheswaran, PhD, of the MGH Cancer Center, co-senior author of the Cell paper. "Our findings that the presence of CTC clusters in the blood of cancer patients is associated with poor prognosis may identify a novel and potentially targetable step in the blood-borne spread of cancer."

In their experiments the team used two versions of a microfluidic device called the CTC-Chip – both developed at the MGH Center for Engineering in Medicine – that captures CTCs from blood samples in ways that make the cells accessible for scientific testing. One version – the ^{HB}CTC-Chip – can efficiently capture extremely rare CTCs in a blood sample. Another version, the CTC-iChip, rapidly isolates CTCs in a way that does not rely on preidentified tumor antigens, allowing capture of cells with gene expression patterns that may be missed by the antibodies used in the ^{HB}CTC-Chip.

A series of experiments in animal models of breast cancer revealed that:

- CTC clusters are made up of cells that probably were adjacent to each other in the primary tumor, not cells that proliferated after entering the bloodstream.
- Although CTC clusters make up only 2 to 5 percent of all CTCs, they contributed to around half of lung metastases resulting from implanted breast tumors, indicating a metastatic potential 23 to 50 times greater than single CTCs.
- CTC clusters injected into mice survived in greater numbers than did single CTCs, and metastases developing from clusters led to significantly reduced survival.
- CTC clusters disappear from the animals' bloodstreams more rapidly than do single CTCs, probably because they become lodged in capillaries where they give rise to metastases.

Analysis of blood samples taken at several points in time from a group of patients with different forms of advanced metastatic breast cancer found CTC clusters in the blood of 35 percent of patients and that the survival of those with more clusters in their blood was significantly reduced. Similar analysis of samples from a group of prostate cancer patients also found an association between the presence of CTC clusters and dramatically reduced survival.

RNA sequencing of both single and clustered CTCs from breast cancer patients identified several genes expressed at elevated levels in CTC clusters, one of which – a protein called plakoglobin – also was overexpressed in the primary tumors of patients with reduced survival. Analysis of blood and tissue samples from one patient revealed that plakoglobin was expressed in CTC clusters but not single CTCs and also was expressed in some portions of both the primary tumor and metastases. Plakoglobin is a component of two important structures involved in cell-to-cell adhesion, and the investigators found that

suppressing its expression caused CTC clusters to fall apart, reducing their metastatic potential, and also disrupted cell-to-cell contact between breast cancer cells but not normal breast tissue.

"It is possible that therapeutically targeting plakoglobin or pathways involved in cell-to-cell adhesion within cancer cells could be clinically useful, especially in patients in whom CTC clusters are found," says Nicola Aceto, PhD, of the MGH Cancer Center and lead author of the Cell paper. "We need to investigate that possibility along with determining whether further characterization of both single CTCs and CTC clusters will provide further insight into differences in their biology, drug responsiveness and their contribution to the process of metastasis."

Provided by Massachusetts General Hospital