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The Opposing Function of Leukocytes in Liver and Lung Metastases

Protsyuk, Darya

Abstract: Metastasis is a step during cancer progression that is clinically challenging to determine and treat. The association of tumor cells with leukocytes influences successful tumor cell seeding and their survival in the metastatic niche. Selectins are vascular adhesion receptors that facilitate the recruitment of leukocytes to metastatic sites, and mediate interactions between tumor and stromal cells. Particularly the absence of L-selectin on leukocytes decreases the infiltration of immune cells in the lungs and reduces pulmonary metastasis. Our aim was to elucidate the contribution of L-selectin during metastatic development in lungs and liver. While pulmonary metastasis was attenuated, liver metastasis was increased in L-selectin deficient mice. We determined that the selectin-dependent recruitment of inflammatory monocytes induced vascular permeability, promoted the extravasation of tumor cells and supported tumor cell survival in the lungs. The adoptive transfer of wild type bone marrow-derived monocytes into L-selectin deficient mice ameliorated metastatic development in the lungs. In the liver, we observed increased amounts of Kupffer and T cells in the absence of L-selectin. Furthermore, L-selectin facilitated the recruitment of neutrophils into the metastatic liver. We showed that liver neutrophils produce reactive oxygen species in vivo, and increased tumor cell apoptosis in vitro. Depleting neutrophils at later metastatic stages increased liver metastasis, confirming the cytotoxic function of neutrophils. In summary, we identified an opposing role of L-selectin on metastatic development in the lungs and the liver. In the lungs, L-selectin mediates the recruitment of monocytes that promote tumor cell extravasation and support metastatic development. Our study demonstrated the contribution of myeloid cell populations to tissue-specific metastasis and defined a novel role of L-selectin during metastatic progression.

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SUMMARY

Metastasis is a step during cancer progression that is clinically challenging to determine and treat. The association of tumor cells with leukocytes influences successful tumor cell seeding and their survival in the metastatic niche. Selectins are vascular adhesion receptors that facilitate the recruitment of leukocytes to metastatic sites, and mediate interactions between tumor and stromal cells. Particularly the absence of L-selectin on leukocytes decreases the infiltration of immune cells in the lungs and reduces pulmonary metastasis. Our aim was to elucidate the contribution of L-selectin during metastatic development in lungs and liver.

While pulmonary metastasis was attenuated, liver metastasis was increased in L-selectin deficient mice. We determined that the selectin-dependent recruitment of inflammatory monocytes induced vascular permeability, promoted the extravasation of tumor cells and supported tumor cell survival in the lungs. The adoptive transfer of wild type bone marrow-derived monocytes into L-selectin deficient mice ameliorated metastatic development in the lungs.

In the liver, we observed increased amounts of Kupffer and $\gamma\delta$ T cells in the absence of L-selectin. These cells were also enriched in adult L-selectin deficient mice that were tumor-free, but their influence on metastatic development was shown to be limited. Furthermore, L-selectin facilitated the recruitment of neutrophils into the metastatic liver. We showed that liver neutrophils produce reactive oxygen species *in vivo*, and increased tumor cell apoptosis *in vitro*. Depleting neutrophils at later metastatic stages increased liver metastasis, confirming the cytotoxic function of neutrophils. Accordingly, the adoptive transfer of wild type neutrophils decreased liver metastasis in L-selectin deficient mice.

In summary, we identified an opposing role of L-selectin on metastatic development in the lungs and the liver. In the lungs, L-selectin mediates the recruitment of monocytes that promote tumor cell extravasation and support metastatic development. In the liver, L-selectin facilitates the recruitment of cytotoxic neutrophils that eliminate tumor cells and decrease metastasis. Our study demonstrated the contribution of myeloid cell populations to tissue-specific metastasis and defined a novel role of L-selectin during metastatic progression.

ZUSAMMENFASSUNG

Metastase ist ein Prozess, der während einer Krebserkrankung stattfindet, die schwer zu bestimmen und behandeln ist. Die Assoziierung von Tumorzellen mit Leukozyten beeinflusst die Verbreitung und das Überleben der Tumorzellen in der metastatischen Umgebung. Selektine sind vaskuläre Adhäsionsrezeptoren, welche die Rekrutierung von Leukozyten zu metastatischen Standorten vereinfachen, und Interaktionen zwischen Tumor- und Stromazellen unterstützen. Vor allem die Absenz von L-Selektin auf Leukozyten vermindert die Infiltration von Immunzellen in den Tumor und die Entwicklung pulmonaler Metastasen. Unser Ziel war es, den Beitrag von L-Selektin zur Metastase in den Lungen und in der Leber zu beschreiben.

In Mäusen ohne L-Selektin gab es weniger pulmonale Metastasen, während eine Zunahme von Metastasen in der Leber beobachtet werden konnte. Wir konnten zeigen, dass in den Lungen, die Selektin-abhängige Rekrutierung von inflammatorischen Monozyten zu einer Zunahme der vaskulären Permeabilität, einer vermehrten Infiltration von Tumorzellen aus der Blutbahn in das unterliegende Gewebe, und verbessertes Überleben der Tumorzellen geführt hat. Der Transfer von Knochenmark-abgeleiteten Monozyten einer wild typ Maus in die Lungen eines L-Selektin Mutanten, hat die metastatische Entwicklung in den Lungen wieder korrigiert.

In der Leber haben wir eine Zunahme an Kupffer und $\gamma\delta$ -T-Zellen in L-Selektin defizitären Mäusen beobachtet. Diese Zunahme war auch in L-Selektin-Mutanten zu beobachten, die nicht mit Tumoren belastet waren. Wir haben gezeigt, dass diese Zellpopulationen während der Metastase fast keine Rolle spielen. Wir konnten aber in vivo zeigen, dass neutrophile Granulozyten reaktive Sauerstoffspezies generieren und dass diese Zellen in vitro zu einer Zunahme der Apoptose in Tumorzellen führen. Wurden neutrophile Granulozyten während der späten Metastase dezimiert, konnten vermehrt Metastasen in der Leber beobachtet werden, welches die zytotoxische Rolle der neutrophile Granulozyten bestätigt.

Zusammenfassend haben wir eine entgegengesetzte Rolle von L-Selektin auf die Metastase in den Lungen und der Leber feststellen können. In den Lungen bewirkt L-Selektin die Rekrutierung von Monozyten, welche die Einwanderung von Tumorzellen und damit die Entwicklung von Metastasen begünstigen. In der Leber fördert L-Selektin die Rekrutierung von zytotoxischen neutrophilen Granulozyten, welche Tumorzellen eliminieren und damit Metastasen vermindern. Unsere Studie demonstriert die Rolle der myeloiden Zellpopulationen während gewebespezifischer Metastase und definiert eine neue Rolle für L-Selektin während dem metastatischen Prozess.