The Role of the Liver Environment in the Regulation of Colon Cancer Metastasis

Isabel Zvibel PhD[1], Yaron Mintz MD[2], Shlomo Brill MD[1], Zamir Halpern MD[1] and Moshe Papa MD[1,2]

[1]Liver Metastasis Research Group, Gastroenterology Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, and [2]Department of Surgical Oncology, Sheba Medical Center, Tel-Hashomer, Israel

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The phenomenon of site-specific metastasis was described as early as 1889, when Paget suggested the "seed and soil" hypothesis [1]. He proposed that for tumor cells to metastasize to a certain organ, that organ must be a "fertile soil" for those tumor cells. The local milieu of the colonized organ can dictate the establishment and growth of tumor cells. In order to continue the metastatic process, after extravasation from circulation, metastatic cells have to recognize and attach to the local extracellular matrix components and to the cells of the organ [2,3]. Soluble paracrine signals secreted by the cells of specific organs can stimulate or inhibit the proliferation of the tumor cells [4–6].

Role of Hepatocyte-derived Extracellular Matrix in Colon Cancer Proliferation

We previously reported that the ability of tumor cells to metastasize *in vivo* to certain organs correlates with the ability of the cells to survive on ECM derived from that organ [2,7]. We found that the ECM components responsible for this effect were heparin proteoglycans and that they acted via regulation of autocrine growth factor expression in the tumor cells [8]. The ECM of the normal liver, found in the space of Disse and secreted by endothelial cells and hepatocytes, consists mainly of collagens type I and type VI, fibronectin and the hepatocyte-specific proteoglycan. Discrete aggregates of type IV collagen and filaments of type V and type III collagen are also detected [9]. One of the unique elements of the hepatic ECM is the cell surface hepatocyte proteoglycan, which belongs to the syndecan family [10]. Unlike proteoglycans from other tissues, its glycosaminoglycan chains are highly sulfated and very similar in their carbohydrate chemistry to mast cell-produced heparin [11]. The main hepatocyte proteoglycan is syndecan-2 or fibroglycan [10], but hepatocytes also express syndecans-1 and -4 [12].

We investigated the effect of hepatocyte-derived ECM on the proliferation of four colon cancer cell lines. These colon cancer cell lines had weak or strong metastasizing potential to the liver. We found that hepatocyte-derived ECM selectively enhances the clonal growth of the strongly metastatic cells, but not of the weakly metastatic ones. The effect of liver ECM on the cell proliferation was, however, uniformly stimulatory of growth in all cell lines [Table 1]. This stimulation of growth was accompanied by changes in the expression of members of the epidermal growth factor family. We found that hepatocyte ECM increased the expression of two members of the EGF family, erb-2 and cripto. By modifying the ECM produced by hepatocytes, we showed that some of these effects were due to the heparin proteoglycans present in the ECM [13].

This effect of hepatocyte ECM on cell proliferation was quite specific, since ECM derived from fibroblasts inhibited the proliferation of the colon cancer cell lines [13]. The ECM deposited by fibroblasts is very different from that secreted by epithelial cells, consisting of collagen type I, fibronectin and chondroitin or dermatan sulfate proteoglycans. We also tested the effects of soluble heparin when the cells were plated on various components found in liver ECM, such as fibronectin, and collagens I and IV. Heparin inhibited growth of the colon cancer cells differently, depending on the matrix component they were plated on and according to their metastatic potential [14]. Moreover, erb-B2 expression was decreased by heparin in the low metastatic cells and increased in the highly metastatic cells when the cells were grown on plastic [14].

Role of Hepatocyte-derived Soluble Factors in Colon Cancer Proliferation

We next investigated the effect of hepatocyte-derived soluble factors on cell proliferation and autocrine growth factor expression in human colon cancer cell lines. We found that hepatocyte-derived soluble factors, produced in co-cultures with the colon cells, as well as hepatocyte-derived conditioned medium, were growth inhibitory for the colon cancer cells [Table 2]. After 4 days in co-cultures with hepatocytes, colon cancer cells showed decreased expression of the EGF receptor family member, erb-B2 [Table 2], as well as decreased expression of heregulin alpha [15]. Hepatocytes are known to produce various growth factors, particularly during liver regeneration, such as transforming

ECM = extracellular matrix components

EGF = epidermal growth factor

Table 1. The effect of hepatocyte-derived ECM on clonal growth, proliferation and erb-b2 expression in colon cancer cell lines

Cell line	Met. potential to the liver	Effect of ECM on: Clonal growth	Cell proliferation	Erb-B2 expression
KM12	Low	No effect	Increase	Increase
SM	High	Increase	Increase	Increase
LS174T	Low	No effect	Increase	Increase
LiM6	High	Increase	Increase	Increase

Table 2. The effect of hepatocyte-derived soluble factors on cell proliferation and erb-B2 expression in colon cancer cell lines

Cell line	Met. potential to the liver	Cell proliferation		Erb-B2 expression	
		Co-culture	Hepatocyte conditioned medium	Co-culture	Hepatocyte conditioned medium
KM12	Low	Decrease	Decrease	Decrease	Decrease
SM	High	Decrease	Decrease	Decrease	Decrease
LS174T	Low	Decrease	Decrease	Decrease	Decrease
LiM6	High	Decrease	Decrease	Decrease	Decrease

growth factor-α, insulin-like growth factors and their binding proteins, etc. [4].

We have yet to identify the active soluble factor produced by the hepatocytes that affects the colon cells. Our results show, paradoxically, that soluble factors secreted by hepatocytes inhibit the proliferation of colon cancer cell lines. However, the liver comprises other cell types, such as Ito cells, Kupffer cells and endothelial cells, that may secrete stimulatory factors for colon cancer cell proliferation.

The EGF Family and its Role in Colon Cancer

The EGF family, both ligands and receptors, are crucial factors for the growth of epithelial tumors, and their expression has been shown to increase in various epithelial tumors, including colorectal carcinomas [16]. The EGF receptor family consists of four known receptors: EGF receptor, or erb-B1, erb-B2, erb-B3 and erb-B4 [17]. The members of this family can form either homo- or hetero-dimers with each other, and can bind and respond to different ligands according to the receptors present in the complex. The known ligands are EGF, TGFα, HB-EGF, betacellulin and AR [17]. Another member of the EGF family is cripto.

The number of EGF receptors as well as c-met was found to correlate with colon cancer metastasis to the liver [18]. Most human colon cancers and cell lines express the EGF receptor ligands TGF-α, amphiregulin, and cripto [16]. Erb-B2, found to be amplified in breast tumors and to correlate with poor prognosis, was not found in normal colon tissue, but its expression was significantly increased in adenomas and adenocarcinomas of the colon [19,20]. Both cripto and amphiregulin were found to be autocrine growth factors for colon carcinoma cell lines, and decreasing their expression or mode of action by antisense RNA or blocking with antibodies reduced tumor cell growth in nude mice and cell proliferation *in vitro* [21,22].

TGF-α = transforming growth factor alpha

Interactions between Colon-derived Autocrine Growth Factors and Hepatocyte ECM

One of the elements modifying the response of cells to a growth factor is the ECM surrounding the cell. Amphiregulin, HB-EGF and heregulins are heparin-binding growth factors, and their effects on a given cell were shown to be modified in the presence of heparin [23]. Amphiregulin, but not HB-EGF, requires cell surface heparin proteoglycans for mitogenic activity [23]. When the colon cells were plated on hepatocyte-derived ECM, both amphiregulin and HB-EGF evoked a response in the cells [24]. Amphiregulin inhibited the growth of the weakly liver-colonizing KM12 cell line, while the strongly metastatic cells KM12SM were stimulated to proliferate [Table 3]. HB-EGF stimulated proliferation of both cell lines. This suggests a possible mechanism for the proliferation of colorectal tumor cells in the liver, with highly metastatic liver-colonizing colon cells being stimulated to proliferate by the synergistic signal of autocrine growth factors and the surrounding hepatic ECM.

Ligands for EGF receptor cause autoinduction of various other members of the EGF family in human keratinocytes, so EGF and TGF-α for example induce expression of themselves as well as expression of HB-EGF and amphiregulin [25,26]. The mechanism is due to stabilization of the mRNA for the growth factors. Indeed, we found that amphiregulin caused an increase in the expression of erb-B2 in colon cell lines grown on plastic and decreased erb-B2 expression when the cells were cultured on ECM [24]. Moreover, amphiregulin increased expression of its own mRNA in the weakly metastatic KM12 and decreased it in the strongly metastatic KM12SM when the cells were cultured on plastic [24].

Expression of ligands of TGF-α and amphiregulin was decreased in both cell lines when the cells were grown on

Table 3. The effect of amphiregulin and HB-EGF on the proliferation of colon cancer cells on hepatocyte-derived ECM

Cell line	Met. potential to the liver	Amphiregulin +ECM	HB-EGF +ECM
KM12	Low	Decrease	Increase
SM	High	Increase	Increase

ECM; however, the expression of cripto was decreased in KM12 and increased in KM12SM cells when the cells were grown on hepatocyte ECM [24]. However, in these studies, there was no correlation between cell proliferation and erb-B2 expression, since amphiregulin stimulated growth of the highly metastatic cells and inhibited the growth of low metastatic cells, and erb-B2 expression was decreased by amphiregulin in both colon cell lines when the cells were grown on ECM. Therefore, erb-B2 is probably not the sole mediator of cell proliferation in these cells.

Probably the other key factor relevant for the effect of hepatocyte-ECM on proliferation of colon cells is cripto. Hepatocyte-derived ECM decreased cripto mRNA levels in the weakly metastatic KM12 and increased cripto in the strongly metastatic cell line KM12SM.

One possibility to explain the varying levels of cripto, AR and TGF-α that did not always correlate with cell proliferation might be that each growth factor affects colon cancer cells differently, depending on the state of differentiation of the cell. In the normal intestine, it was demonstrated that TGF-α has a different effect and distribution in the stem cells at the base of the cript than in the more mature, differentiated cells at the tip of the villus. As they move towards the tip of the villus and differentiate, both goblet cells and enterocytes lose their ability to respond to TGF-α with a proliferative response [27].

Mechanism of Action of Proteoglycans

Conrad and associates [28] have shown that a highly sulfated heparan sulfate species produced by growth-arrested hepatocytes directly inhibits proliferating hepatocytes by being internalized to the nucleus. Heparin and heparan sulfate have been found to stimulate or inhibit cell proliferation by several mechanisms, depending on the cell type. Effects of heparin on the number of cell surface receptors, as we observed with erb-B2, were seen in smooth muscle cells. In these cells, heparin reduced the number of EGF receptors [29] and therefore inhibited cell growth. On the other hand, endothelial cells were stimulated to proliferate by heparin, which acted synergistically with bFGF [30].

Many of the effects of heparin and heparan sulfate are mediated via high affinity binding of growth factors. Recently, specific residues have been identified that are responsible for binding hepatocyte growth factor and bFGF [31,32]. Tissue-specific heparins, acting synergistically with circulating hormones, can also regulate mRNA synthesis and abundance of tissue-specific genes in primary cultures of hepatocytes [33]. The transcriptional effect of heparin was also shown in other cell systems and affected the expression of matrix-degrading enzymes or early response genes, such as jun and fos [34].

bFGF = basic fibrocyte growth factor

Conclusions

The final fate of the colon cancer cell metastasizing to the liver will be determined by the interplay between the stimulatory effect of the ECM, the effect of the cell-derived soluble factors and the response of the colon cancer cells. This response is dictated by the state of differentiation of the colon cell, by the autocrine growth factors and receptors expressed, and by the chemistry of the surrounding ECM.

References

1. Paget S. The Lancet 1889;i:571.
2. Zvibel I, Kraft A. Extracellular matrix and metastasis. In: Zern MA, Reid LM, eds. Extracellular Matrix: its Chemistry, Biology, and Pathobiology. Vol. 22. New York: Marcel Dekker Inc., 1993:559–80.
3. Nicolson GL. Metastatic tumor cell interactions with endothelium, basement membrane and tissue. *Curr Opin Cell Biol* 1989;1:1009–19.
4. Radinsky R. Paracrine growth regulation of human colon carcinoma organ-specific metastasis. *Cancer Metastasis Rev* 1993;12:345–61.
5. Nicolson GL, Dulski KM. Organ specificity of metastatic tumor colonization is related to organ-selective growth properties of malignant cells. *Int J Cancer* 1986;38:289–94.
6. Szaniawska B, Majewski S, Kaminski MJ, Noremberg K, Swierz M, Janik P. Stimulatory and inhibitory activities of lung-conditioned medium on the growth of normal and neoplastic cells in vitro. *J Natl Cancer Inst* 1985;75:303–6.
7. Doerr R, Zvibel I, Chiuten D, D'Olimpio J, Reid LM. Clonal growth of tumors on tissue-specific biomatrices and correlation with organ-site specificity of metastases. *Cancer Res* 1989;49:384–92.
8. Zvibel I, Brill S, Halpern Z, Papa M. Hepatocyte extracellular matrix modulates expression of growth factors and growth factor receptors in human colon cancer cells. *Exp Cell Res* 1998;245:123–31.
9. Martinez-Hernandez A, Amenta PS. Morphology, localization, and cellular origin of hepatic extracellular matrix. In: Zern MA, Reid LM, eds. Extracellular Matrix: Its Chemistry, Biology, and Pathobiology. Vol. 22. New York: Marcel Dekker Inc., 1993:201–55.
10. Pierce A, Lyon M, Hampson IN, Cowling GJ, Gallagher JT. Molecular cloning of the major cell surface heparan sulfate proteoglycan from rat liver. *J Biol Chem* 1992;267:3894–900.
11. Lyon M, Deakin JA, Gallagher JT. Liver heparan sulfate structure. *J Biol Chem* 1994;269:11208–15.
12. Kim CW, Goldberger OA, Gallo RL, Bernfield M. Members of the syndecan family of heparan sulfate proteoglycans are expressed in distinct cell-, tissue- and development-specific patterns. *Mol Biol Cell* 1994;5:797–805.
13. Zvibel I, Halay E, Reid LM. Heparin and hormonal regulation of mRNA synthesis and abundance of autocrine growth factors: relevance to clonal growth of tumors. *Mol Cell Biol* 1991;11:108–16.
14. Zvibel I, Halpern Z, Papa M. Extracellular matrix modulates the expression of growth factors and growth factor receptors in liver-colonizing colon cell lines. *Int J Cancer* 1998;77:295–301.
15. Zvibel I, Brill S, Papa M, Halpern Z. Hepatocyte-derived soluble factors regulate proliferation and autocrine growth factor expression in colon cancer cell lines of varying liber-colonizing capability. *Tumor Biol* (in press).
16. Ciardello F, Kim N, Saeki T, Dono R, Persico MG, Plowman GD, Garrigues J, Radke S, Todaro GJ, Salomon DS. Differential expression of epidermal growth factor-related proteins in human colorectal tumors. *Proc Natl Acad Sci USA* 1991;88:7792–6.
17. Beerli RR, Hynes NE. Epidermal growth factor-related peptides activate distinct subsets of erbB receptors and differ in their biological activities. *J Biol Chem* 1996;271: 6071–6.
18. Radinsky R. Molecular mechanisms for organ-specific colon carcinoma metastasis. *Eur J Cancer* 1995;31A:1091–5.
19. Kapitanovic S, Radosevic S, Kapitanovic M, Andelinovic S, Ferencic Z, Tavassoli M, Primorac D, Sonicki Z, Spaventi S, Pavelic K, Spaventi R. The expression of p185 (HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. *Gastroenterology* 1997;112:1103–13.
20. SaekiT, Salomon DS, Johnson GR, Gullick WJ, Mandai K, Yamagami K,

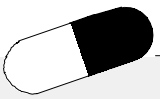
Moriwaki S, Tanada M, Takashima S, Tahara E. Association of epidermal growth factor-related peptides and type I receptor tyrosine kinase receptors with prognosis of human colorectal carcinomas. *Jpn J Clin Oncol* 1995;25:240–9.

21. Ciardello F, Tortora G, Bianco C, Selvam MP, Basolo F, Fontanini G, Pacifico F, Normanno N, Brandt R, Persico GM, Salomon DS, Bianco AR. Inhibition of cripto expression and tumorigenicity in human colon cancer cells by antisense RNA and oligodeoxynucleotides. *Oncogene* 1994;9:291–8.
22. Johnson GR, Saeki T, Gordon AW, Shoyab M, Salomon DS, Stromberg K. Autocrine action of amphiregulin in a colon carcinoma cell line and immunochemical localization of amphiregulin in human colon. *J Cell Biol* 1992;118:741–51.
23. Johnson GR, Wong L. Heparan sulfate is essential to amphiregulin-induced mitogenic signaling by the epidermal growth factor receptor. *J Biol Chem* 1994;269:27149–54.
24. Zvibel I, Brill S, Halpern Z, Papa MZ. Amphiregulin and hepatocyte-derived extracellular matrix regulate proliferation and autocrine growth factor expression in colon cancer cell lines of varying liver-colonizing capability. *J Cell Biochem* (in press).
25. Barnard JA, Graves-Deal R, Pittelkow LR, DuBois R, Cook P, Ramsey GW, Bishop PR, Damstrup L, Coffey RJ. Auto- and cross-induction within the mammalian epidermal growth factor-related peptide family. *J Biol Chem* 1994;269:22817–22.
26. Stoll S, Garner W, Elder J. Heparin-binding ligands mediate autocrine epidermal growth factor receptor activation in skin organ culture. *J Clin Invest* 1997;100:1271–81.
27. Huang F, Sauma S, Yan Z, Friedman E. Colon absorptive cells lose proliferative response to TGF α as they differentiate. *Exp Cell Res* 1995;219:8–14.
28. Fedarko NS, Conrad HE. A unique heparan sulfate in the nuclei of hepatocytes: structural changes with the growth state of the cells. *J Cell Biol* 1986;102:587–99.
29. Reilly CF, Fritze MS, Rosenberg RD. Antiproliferative effects of heparin on vascular smooth muscle cells are reversed by epidermal growth factor. *J Cell Physiol* 1987;121:149–57.
30. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell* 1991;64:841–8.
31. Lyon M, Deakin JA, Mizuno K, Nakamura T, Gallagher JT. Interaction of hepatocyte growth factor with heparan sulfate. Elucidation of the major heparan sulfate structural determinants. *J Biol Chem* 1994;269:11216–23.
32. Turnbull JE, Fernig DG, Ke Y, Wilkinson MC, Gallagher JT. Identification of the basic fibroblast growth factor binding sequence in fibroblast heparan sulfate. *J Biol Chem* 1992;267:10337–41.
33. Spray DC, Fujita M, Saez JC, Choi H, Watanabe T, Hertzberg EL, Rosenberg LC, Reid LM. Glycosaminoglycans and proteoglycans induce gap junction synthesis and function in primary liver cultures. *J Cell Biol* 1987;105:541–51.
34. Busch SJ, Jackson RL, Sassone-Corsi P. Heparin inhibits transcriptional activation by Fos and Jun oncoproteins. *Trans Assoc Am Phys* 1990;103:21–7.

Correspondence: Dr. I. Zvibel, Liver Metastasis Research Group, Gastroenterology Institute, Tel Aviv Sourasky Medical Center, 6 Weizmann St., Tel Aviv 64239, Israel. Tel: (972-3) 697 4481; Fax: (972-3) 697 4622; email: isab@tasmc.health.gov.il

Everything should be made as simple as possible, but no simpler.
Albert Einstein

Capsule



Alcohol and coronary heart disease

The association between alcohol consumption and risk of cardiovascular outcomes in diabetic individuals has not been determined. A group from the University of Wisconsin tried to examine the relationship between alcohol intake and coronary heart disease (CHD) mortality in persons with older-onset diabetes.

In a population-based prospective cohort study conducted from 1984 through 1996 with a follow-up of up to 12.3 years, 983 older-onset diabetic individuals were interviewed about their past-year intake of alcoholic beverages. The main outcome measure was time to mortality from CHD by category alcohol intake.

The results showed that alcohol use was inversely associated with risk of CHD mortality in older-onset diabetic subjects. The CHD mortality rates for never and former drinkers were 43.9 and 38.5 per 1,000 person-years, respectively, while the rates for those with alcohol intakes of less than 2, 2 to 13, and 14 or more g/day were 25.3, 20.8, and 10.0 per 1,000 person-years, respectively. Compared with never-drinkers, and controlling for age, gender, cigarette smoking, glycosylated hemoglobin level, insulin use, plasma C-peptide level, history of angina or myocardial infarction, digoxin use, and the presence and severity of diabetic retinopathy, former drinkers had a relative risk of 0.69 (95% confidence interval, 0.43–1.12). For those who drank less than 2 g/day (less than 1 drink a week), the RR was 0.54 (95% CI, 0.33–0.90); for 2 to 13 g/day it was 0.44 (95% CI, 0.23–0.84); and for 14 or more g/day (about 1 drink or more a day) it was 0.21(95% CI, 0.09–0.48). Further adjustments for blood pressure, body mass index, education, physical activity, diabetes duration, hypertension history, overt nephropathy, peripheral neuropathy, lipid measures, or intake of medications such as aspirin and anti-hypertensive agents, did not change the associations observed. The authors conclude that there is an overall beneficial effect of alcohol consumption in decreasing the risk of death due to CHD in people with older-onset diabetes.

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