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Mini Review

Mechanisms behind signet ring cell carcinoma formation

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ABSTRACT

Signet ring cell carcinomas are highly malignant dedifferentiated adenocarcinomas. There are no cell–cell interactions between these round-shaped cells. They contain huge numbers of vacuoles, filled with mucins, which are secreted from the cells. The mechanism behind this phenotype has recently begun to be elucidated. In highly differentiated adenocarcinomas the ErbB2/ErbB3 complex is activated, which is followed by phosphatidylinositol 3-kinase (PI3K) activation. p38 MAP kinase is activated downstream of PI3K and adherens junctions are disrupted via Rac1 activation. Loss of adherens junctions leads to the disappearance of tight junctions, which results in a loss of cell–cell interactions. Secretion of mucin is enhanced by activation of PI3K. One of the mucins – Muc4 – can activate ErbB2. Under normal conditions Muc4 and ErbB2 are separated by adherens and tight junctions, however in signet ring cells they are able to interact, since these junctions have been lost. Therefore, an activation loop is formed, consisting of ERbB2/ErbB3–Muc4–ErbB2/ErbB3. As a result, the ErbB2/ErbB3 signaling pathway becomes constitutively activated, cell–cell interactions are lost, and signet ring carcinomas are formed. As a result of constitutive activation of the ErbB2/ErbB3 complex, cell growth is continuously enhanced. Some signet ring cell carcinomas have been found to have mutations in the E-cadherin gene, which fits the above hypothesis.

1. Introduction

Dedifferentiated carcinoma often brings the worst prognosis for patients due to its aggressive and infiltrative nature, with desmoplastic reactions making surgical removal difficult [1]. These carcinoma cells lack the ability to maintain cell–cell contact, and therefore diffusely infiltrate the stroma, resulting in increased invasion and metastasis. Signet ring cells exhibit round shapes with eccentric nuclei and have abundant muscus granules in the cytoplasm, which secrete mucins. Signet ring cell carcinomas are one of the most malignant cancers. Signet-ring cells are most frequently associated with stomach cancer [2], but are observed in many tissues including the prostate [3], bladder, gallbladder [4], breast, and colon [5], as well as in stromal tumors of the ovary and testis [6].

The mechanism behind the formation of signet ring carcinomas has recently begun to be elucidated. This review discusses this mechanism in detail.

2. Finding HCC2998 cells opened the door

HCC2998 is a highly differentiated colon adenocarcinoma cell line [7]. When injected into nude mice, these cells form differentiated tumors [8]. When constitutively active PI3K was expressed in these cells, they were converted to a signet ring carcinoma-like cell line with no cell–cell interactions, round cell shape, and abundant secretion of mucins (Fig. 1A) [8]. These cells formed highly invasive dedifferentiated tumors in nude mice [8]. The phenotypes of these cell lines were very similar to those of signet ring carcinomas. Therefore, the status of PI3K was tested in signet ring cell lines established from native signet ring cell carcinomas. In 4 out of 6 cell lines, PI3K was bound to a 200 kb protein, which was phosphorylated at tyrosine residues and possibly activated. This 200 kb protein was identified as ErbB3 [9]. It is well known that ErbB3 can activate PI3K. ErbB2 was also phosphorylated at tyrosine residues in these cells [9]. It has been shown that ErbB3 must be complexed with another EGF family tyrosine kinase to be activated. These results suggest that the ErbB2/ErbB3 complex is constitutively activated in many signet ring cell carcinomas.

Important downstream factors were determined using various inhibitors in the HCC2998 system. Among these, p38 MAP kinase inhibitors blocked the transition of highly differentiated adenocarcinoma cells to poorly differentiated signet ring cell carcinomas [10]. Dominant active MKK6 (which lies upstream of p38 MAP kinase) was expressed. Cells lost cell–cell interactions and became signet ring cell-like, however, enhancement of mucin secretion was not observed. This suggests that cell–cell interactions and secretion of mucin are regulated by different signaling pathways

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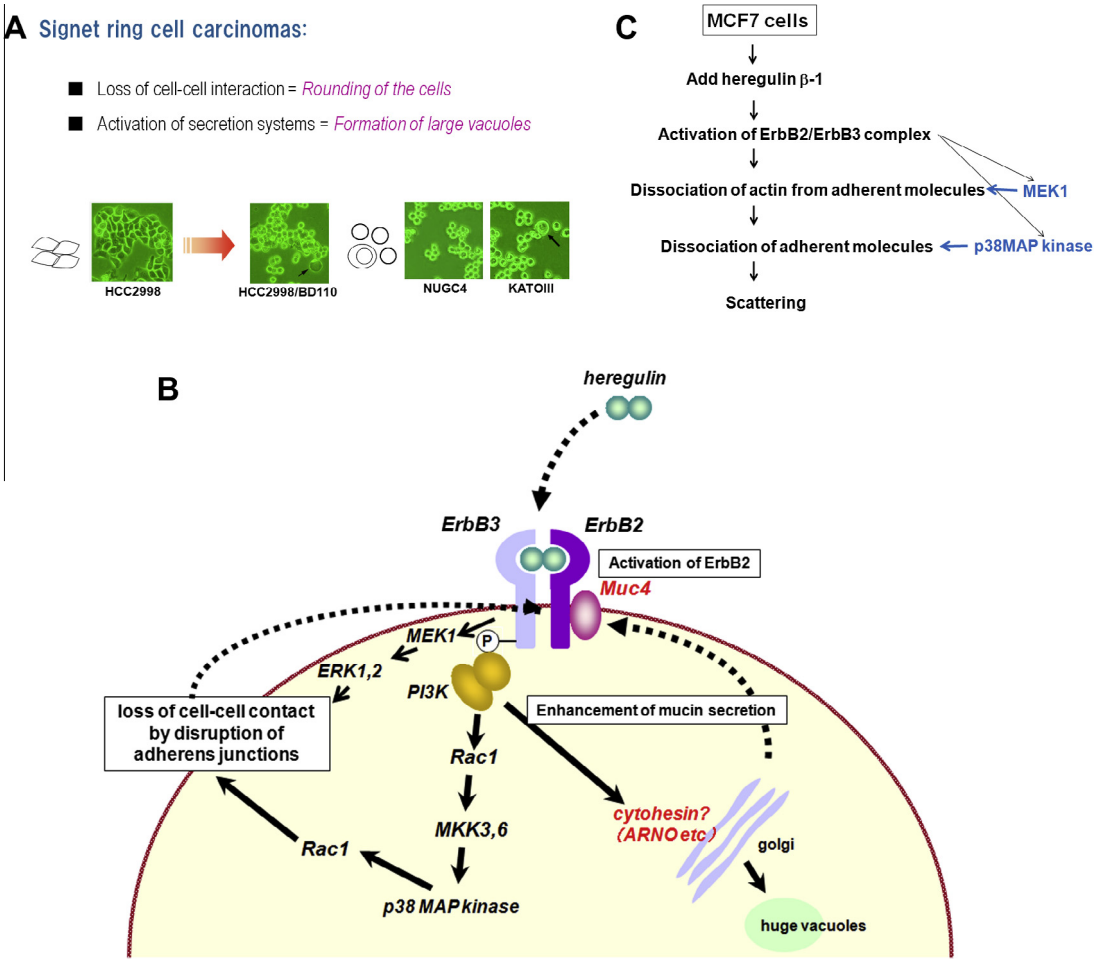

Fig. 1. (A) Highly differentiated adenocarcinoma HCC2998 cells can be converted to signet ring-like cells after expression of constitutively active PI3K. NUGC4 and KATOIII cells are established from signet ring cell carcinomas [22,23]. These cells are quite similar to the cells yielded by expression of constitutively activated PI3K in HCC2998 cells. Arrows indicate signet ring cells. (B) A model for signaling in signet ring cell carcinomas. The ErbB2/ErbB3 pathway is important for signaling of signet ring cell carcinomas. Both the MEK1 and p38 MAP kinase pathways contribute to dissociation of the cells. Secretion of Muc4, which activates ErbB2, is enhanced due to the activation of PI3K. This creates an activation loop of ErbB2/ErbB3–Muc4–ErbB2/ErbB3. Overall, if there is a trigger for activation of these pathways, and if cells are basically transformed, signet ring cell carcinomas will be formed. (C) Signaling pathways for dissociation of the cells. Activation of the MEK1 pathway is required for dissociation of F-actin from the adherens and tight junction complexes. After this, p38 MAP kinase will activate Rac1 to dissociate adherens junctions and cells will be completely dispersed.

[10]. Indeed p38 MAP kinase was constitutively activated in signet ring cell lines [10].

It has been suggested that Muc4 binds and activates ErbB2 [11]. This binding interaction was observed in a signet ring carcinoma cell line [12]. Inhibiting the secretion of Muc4 blocked activation of ErbB2 [12]. Therefore, Muc4 was shown to play an important role in maintenance of the malignant phenotype. However, in normal cells, Muc4 resides on the apical membrane, while ErbB2 is found on the basolateral membrane, such that the two never meet. Because signet ring cell carcinomas have lost the barriers of adherens and tight junctions, these two proteins are then able to interact (Fig. 1B).

3. MCF7-Heregulinβ-1(HRG) system reveals loss of cell–cell interactions

It has been shown that MCF7 cells undergo scattering after stimulation with HRG [13,14]. Scattering has been well studied using EGF and other growth factors [15,16]. It has been shown that scattering is mediated by the p38 MAP kinase -Rac1 pathway [17,18], which disrupts adherens junctions. Indeed expression of dominant active MKK6 results in activation of Rac1 and disruption of adherens junctions [18]. However, these cells do not disperse completely, but instead attach to each other within a small region. This is due to F-actin, which does not detach from adherens junction complexes, leading to incomplete disruption of these junctions [18]. The major pathways that the ErbB2/ErbB3 complex activates are the PI3K and the ERK1, 2 pathways. Careful examination of scattering caused by HRG revealed that F-actin disappears from adherens junctions first (Fig. 1C). Inhibition of the MEK1 pathway blocks scattering as well [19]. In this case, F-actin does not disappear from adherens junctions. These results suggest that activation of the MEK1 pathway is required to dissociate F-actin from adherens junctions. Indeed expression of both dominant active MKK6 and dominant active MEK1 results in complete dissociation of the cells to yield single cells (Fig. 1C) [18]. Therefore, these two pathways are sufficient to disperse the cells. This is true in the case of MCF7 cells. The mechanism by which HCC2998 cells become signet ring cells is currently unknown. Because these cells do not move and pile up, an analysis is relatively difficult [20]. Additional studies will be required to further clarify this data. In signet ring cells, the ErbB2/ErbB3 pathway may be activated. Therefore, the MEK pathway may be activated to complete the cell–cell dissociation (Fig. 1B).

Scattering is a phenomenon seen in some of the cells. There are some cells that respond to HRG by scattering. Scattering can be

separated into two parts; the enhancement of cell movement and the dissociation of cells [14]. There are some cells that do not scatter after HRG stimulation, despite expressing ErbB2 and ErbB3. HCC2998 cells dissociate from other cells to yield single cells, but cell movement is not enhanced. However, it is quite likely that formation of signet ring cell carcinomas is the result of a scattering response of highly differentiated adenocarcinomas, since HCC2998 cells can be converted to signet ring carcinoma cells through continuous activation of ErbB2/ErbB3 signaling.

HRG-induced scattering of MCF7 cells is highly reversible. As soon as the signaling for scattering stops, cells immediately re-aggregate [19]. Therefore, continuous activation of the signal is necessary to maintain the dispersed phenotype of signet ring cells. In cancer cells, the signals for maintaining the transformed phenotype are usually on continuously, supporting the above hypothesis. This is demonstrated by the activation loop of ErbB2/ErbB3–Muc4–ErbB2/ErbB3 shown above (Fig. 1B). We propose here that the dispersed phenotype of signet ring cells is similar to the scattering of MCF7 cells.

4. Point mutations found in E-cadherin in signet ring carcinomas support this hypothesis

Recently, a mutation in the E-cadherin gene was found in a signet ring carcinoma [21]. The mutation disrupts the adherens junction, making it possible for ErbB2 and Muc4 to interact. This results in creation of the ErbB2/ErbB3–Muc4–ErbB2/ErbB3 activation loop (Fig. 1B), leading to the formation of signet ring carcinomas. In other signet ring carcinomas, similar mechanisms may be found.

5. Conclusion

Formation of signet ring carcinomas must be supported by a basic transformation of the cells. For instance, HCC2998 cells are already transformed. The mechanism mentioned above adds a malignant phenotype. Constitutive activation of the ErbB2/ErbB3 pathway alone may not be sufficient to form signet ring carcinomas. It has been shown that EGF or other growth factors can induce scattering in some cell lines [15,16]. However, the EGF receptor pathway may not cause signet ring cell carcinomas since EGF receptors are often activated in non-signet ring cell carcinomas [8]. It is currently unknown why the ErbB2/ErbB3 pathway is important for signet ring cell carcinomas. It may be because ErbB3 strongly activates PI3K. Further study may be required to elucidate the precise mechanism behind this phenotype.

In conclusion, signet ring cell carcinomas contain basic transformation mutations in addition to ErbB2/ErbB3 pathway activation, adding to the malignant phenotype.

References

[1] N.E. Fusenig, D. Breitkreutz, P. Boukamp, P. Tomakidi, H.J. Stark, Differentiation and tumor progression, Recent Results Cancer Res. 139 (1995) 1–19.
[2] H.M. el-Zimaity, K. Itani, D.Y. Graham, Early diagnosis of signet ring cell carcinoma of the stomach: role of the Genta stain, J. Clin. Pathol. 50 (1997) 867–868.
[3] F.J. Leong, A.S. Leong, J. Swift, Signet-ring carcinoma of the prostate, Pathol. Res. Pract. 192 (1996) 1232–1238. discussion 1239–1241.
[4] V. Jain, K. Gupta, R. Kudva, G.S. Rodrigues, A case of ovarian metastasis of gallbladder carcinoma simulating primary ovarian neoplasm: diagnostic pitfalls and review of literature, Int. J. Gynecol. Cancer 16 (Suppl. 1) (2006) 319–321.
[5] T. Makino, T. Tsujinaka, H. Mishima, M. Ikenaga, T. Sawamura, S. Nakamori, K. Fujitani, M. Hirao, M. Kashiwazaki, N. Masuda, M. Takeda, M. Mano, Primary signet-ring cell carcinoma of the colon and rectum: report of eight cases and review of 154 Japanese cases, Hepatogastroenterology 53 (2006) 845–849.
[6] M. Michal, O. Hes, D.V. Kazakov, Primary signet-ring stromal tumor of the testis, Virchows Arch. 447 (2005) 107–110.
[7] M.C. Alley, C.M. Pacula-Cox, M.L. Hursey, L.R. Rubinstein, M.R. Boyd, Morphometric and colorimetric analyses of human tumor cell line growth and drug sensitivity in soft agar culture, Cancer Res. 51 (1991) 1247–1256.
[8] M. Kobayashi, S. Nagata, T. Iwasaki, K. Yanagihara, I. Saitoh, Y. Karouji, S. Ihara, Y. Fukui, Dedifferentiation of adenocarcinomas by activation of phosphatidylinositol 3-kinase, Proc. Natl. Acad. Sci. USA 96 (1999) 4874–4879.
[9] M. Kobayashi, A. Iwamatsu, A. Shinohara-Kanda, S. Ihara, Y. Fukui, Activation of ErbB3-PI3-kinase pathway is correlated with malignant phenotypes of adenocarcinomas, Oncogene 22 (2003) 1294–1301.
[10] Q. Xu, Y. Karouji, M. Kobayashi, S. Ihara, H. Konishi, Y. Fukui, The PI3-kinase-Rac-p38 MAP kinase pathway is involved in the formation of signet-ring cell carcinoma, Oncogene 22 (2003) 5537–5544.
[11] K.L. Carraway, G. Theodoropoulos, G.A. Kozloski, C.A. Carothers Carraway, Muc4/MUC4 functions and regulation in cancer, Future Oncol. 5 (2009) 1631–1640.
[12] A. Yokoyama, B.-H. Shi, T. Kawai, H. Konishi, R. Ando, H. Tachikawa, S. Ihara, Y. Fukui, Muc4 is required for activation of ErbB2 in signet ring carcinoma cell lines, Biochem. Biophys. Res. Commun. 355 (2007) 200–203.
[13] J. Russo, H.D. Soule, C. McGrath, M.A. Rich, Reexpression of the original tumor pattern by a human breast carcinoma cell line (MCF-7) in sponge culture, J. Natl. Cancer Inst. 56 (1976) 279–282.
[14] R. Vadlamudi, L. Adam, A. Talukder, J. Mendelsohn, R. Kumar, Serine phosphorylation of paxillin by heregulin-beta1: role of p38 mitogen activated protein kinase, Oncogene 18 (1999) 7253–7264.
[15] L.J. McCawley, P. O'Brien, L.G. Hudson, Epidermal growth factor (EGF)- and scatter factor/hepatocyte growth factor (SF/HGF)- mediated keratinocyte migration is coincident with induction of matrix metalloproteinase (MMP)-9, J. Cell. Physiol. 176 (1998) 255–265.
[16] M.A. Deugnier, M.M. Faraldo, B. Janji, P. Rousselle, J.P. Thiery, M.A. Glukhova, EGF controls the in vivo developmental potential of a mammary epithelial cell line possessing progenitor properties, J. Cell Biol. 159 (2002) 453–463.
[17] N. Akhtar, N.A. Hotchin, RAC1 regulates adherens junctions through endocytosis of E-cadherin, Mol. Biol. Cell 12 (2001) 847–862.
[18] Y.T. Chang, C.L. Shu, Y. Fukui, Activation of the MEK pathway is required for complete scattering of MCF7 cells stimulated with heregulin-beta1, Biochem. Biophys. Res. Commun. 433 (2013) 311–316.
[19] R. Okoshi, C.L. Shu, S. Ihara, Y. Fukui, Scattering of MCF7 cells by heregulin ss-1 depends on the MEK and p38 MAP kinase pathway, PLoS ONE 8 (2013) e53298.
[20] R. Okoshi, C.L. Shu, S. Ihara, Y. Fukui, Heregulin beta-1 induces loss of cell-cell contact and enhances expression of MUC1 at the cell surface in HCC2998 and MKN45-1 cells, PLoS ONE 6 (2011) e29599.
[21] L.E. Hamilton, K. Jones, N. Church, S. Medlicott, Synchronous appendiceal and intramucosal gastric signet ring cell carcinomas in an individual with CDH1-associated hereditary diffuse gastric carcinoma: a case report of a novel association and review of the literature, BMC Gastroenterol. 13 (2013) 114.
[22] M. Maehara, M. Yagita, Y. Isobe, T. Hoshino, G. Nakagawara, Dimethyl sulfoxide (DMSO) increases expression of sialyl Lewis x antigen and enhances adhesion of human gastric carcinoma (NUGC4) cells to activated endothelial cells, Int. J. Cancer 54 (1993) 296–301.
[23] T. Dohi, T. Nemoto, S. Ohta, K. Shitara, N. Hanai, E. Nudelman, S. Hakomori, M. Oshima, Different binding properties of three monoclonal antibodies to sialyl Le(x) glycolipids in a gastric cancer cell line and normal stomach tissue, Anticancer Res. 13 (1993) 1277–1282.