Molecular Biology of the Cell
Vol. 21, 499–500, February 15, 2010

RETROSPECTIVE

Remembrance of Dead Cells Past: Discovering That the Extracellular Matrix Is a Cell Survival Factor

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Submitted October 27, 2009; Accepted November 16, 2009

In 1992, Jere Meredith and I followed up on a serendipitous observation and showed that matrix deprivation can lead to apoptosis. Our article in *Molecular Biology of the Cell*, together with work form Steve Frisch's lab, helped establish the paradigm that integrin signals control cell survival in a variety of systems. It has been a pleasure to watch that work take on a life of its own as other investigators have explored its role in processes such as cavitation, regression of the mammary gland at the end of pregnancy, cancer metastasis, and tumor resistance to chemotherapy. Recently, we described an exception to the paradigm: In some tumors, reagents that activate integrin signaling enhance apoptosis in response to chemotherapy.

In fall of 1992, I was still working at the bench, measuring intracellular calcium in human endothelial cells as they spread on fibronectin (Schwartz, 1993). It was convenient to detach the cells and keep them in suspension in nonadhesive dishes as a stock for multiple replating assays. However, one time I was lazy or forgetful and left a dish in the incubator overnight. The next morning, I thought they might still be usable, but one look in the microscope showed cells with condensed nuclei and big membrane blebs, about as dead as cells get.

Around this time, "programmed cell death" was becoming a hot area. In particular, there were papers every month about growth factor deprivation triggering this type of cell death. It seemed pretty obvious to me that matrix deprivation should have effects similar to growth factor deprivation. So I thought it might be worth documenting. I asked a recently arrived postdoc, Jere Meredith, if he wanted to look into it as a side project.

Jere is an exceptionally capable guy, and he quickly finished the assays to show that it was apoptotic death. He also showed that it was specifically mediated by integrins and that it occurred in some cell types but not others. Finally, in a gesture to the FAKists who had just recently identified this integrin-regulated tyrosine kinase, he showed that a phosphatase inhibitor blocked cell death under these conditions. That seemed important because it showed that it was a regulated effect. The experimental work took ~8 mo. When I contacted Jere in preparation for this essay, he wrote that it was among the most satisfying experiences of his career: the experiments worked, the results made sense, and the pieces all fit together without much effort.

We thought it was a solid finding with a clear message. *Molecular Biology of the Cell* (*MBoC*) was brand new but had a reputation for quick review and showed signs of being an up-and-coming journal. The manuscript was rapidly accepted with only minor revisions and published a few months after (Meredith *et al.,* 1993). I never seriously considered pursuing the project further. Apoptosis was not my field, and although I saw the potential importance this mechanism might have in vivo, we were working on other things that interested us more.

The following year, Steve Frisch published a very nice paper in the *Journal of Cell Biology* that reported essentially the same effect in MDCK cells and named the phenomenon anoikis (Frisch and Francis, 1994). Steve later told me that he submitted his manuscript a bit before we did but had a long review process. Still, his article has received quite a lot of attention and a larger number of citations. If only I had thought to make up a new name for detachment-induced cell death!

Together, these two articles firmly established the idea that integrin signals control cell survival in a variety of systems. Later in 1994, I was surprised and very pleased to hear that our article had won the *MBoC* Paper of the Year award. The prize included a trip for Jere to the ASCB meeting in December to present the data at a minisymposium. I think he also got a free lunch, but I'm not sure because I wasn't invited.

Papers published in the following few years identified several different pathways that mediated adhesion-dependent survival, including PI 3-kinase and focal adhesion kinase (Frisch *et al.,* 1996; Hungerford *et al.,* 1996; Khwaja *et al.,* 1997). But what has brought the most personal gratification has been watching this small discovery move into the wider world of biology. Within the next few years, integrin regulation of cell survival was found to be important for cavitation, in which epithelial cells form hollow tubes or spheres (Coucouvanis and Martin, 1995). It was implicated in regression of the mammary gland at the end of pregnancy, where cell death is triggered by secretion of matrix-degrading proteases (Wiesen and Werb, 2000). More recently, the mechanistic basis for these effects has been elucidated in some

DOI: 10.1091/mbc.E09–07–0602
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detail, most prominently by the Brugge and Weaver labs, who have identified a network of overlapping molecular mechanisms that contribute to hollowing out of epithelial structures (Mailleux *et al.*, 2008). Loss of this matrix requirement has been linked to cancer metastasis (Simpson *et al.*, 2008), as originally proposed by Frisch and coworkers (Frisch and Francis, 1994). Finally, clinical relevance entered the picture with the discovery that altered matrix composition and integrin signaling in cancer contributes to radiation and chemotherapy resistance (Hodkinson *et al.*, 2007). Indeed, integrin inhibitors enhance therapeutic responses in animal models of cancer and such approaches have been proposed for use in patients (Ning *et al.*, 2008).

That this work has transitioned to the status of paradigm was brought home to me in an interesting way a few years ago. My lab has identified an exception to this principle: In a distinct subset of cancers, including most melanomas, the ability of chemotherapy and radiation to induce apoptosis is *increased* in adherent compared with suspended cells (Lewis *et al.*, 2002; Truong *et al.*, 2003; Schwartz *et al.*, 2008). We originally proposed that this effect might confer therapy resistance to metastatic cells that escaped the tumor and were present in the circulation at the time of therapy (Lewis *et al.*, 2002). But subsequent experiments showed that melanomas, which had previously been found to contain little ECM within tumor micromasses, are protected from chemotherapy by this loss of matrix within the primary tumor (Schwartz *et al.*, 2008). Thus, treating tumor-bearing mice with reagents that activate integrin signaling enhanced responses to chemotherapy. However, these findings have been received the usual skepticism that greets experimental results that contradict established paradigms. On several occasions I've been asked, hadn't I heard that integrins promote survival?

Which is not a bad thing. Scientific work of any importance acquires a life of its own. It was a surprise the first time it happened, but I think I also experienced something like the parental pride that that comes when our children accomplish something that exceeds our own abilities. My lab has probably received more recognition than we deserved for what was really just following a simple observation. It's been a profound pleasure to see it grow up and succeed in the larger world all on its own.

REFERENCES

Coucouvanis, E., and Martin, G. R. (1995). Signals for death and survival: a two-step mechanism for cavitation in the vertebrate embryo. Cell *83*, 279–287.

Frisch, S. M., and Francis, H. (1994). Disruption of epithelial cell-matrix interactions induces apoptosis. J. Cell Biol. *124*, 619–626.

Frisch, S. M., Vuori, K., Ruoslahti, E., and Chan, P. Y. (1996). Control of adhesion dependent cell survival by focal adhesion kinase. J. Cell Biol. *134*, 793–799.

Hodkinson, P. S., Mackinnon, A. C., and Sethi, T. (2007). Extracellular matrix regulation of drug resistance in small-cell lung cancer. Int. J. Radiat. Biol. *83*, 733–741.

Hungerford, J. E., Compton, M. T., Matter, M. L., Hoffstrom, B. G., and Otey, C. A. (1996). Inhibition of pp125FAK in cultured fibroblasts results in apoptosis. J. Cell Biol. *135*, 1383–1390.

Khwaja, A., Rodriguez-Viciana, P., Wennstrom, S., Warne, P. H., and Downward, J. (1997). Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. EMBO J. *16*, 2783–2793.

Lewis, J. M., Truong, T. N., and Schwartz, M. A. (2002). Integrins regulate the apoptotic response to DNA damage through modulation of p53. Proc. Natl. Acad. Sci. USA *99*, 3627–3632.

Mailleux, A. A., Overholtzer, M., and Brugge, J. S. (2008). Lumen formation during mammary epithelial morphogenesis: insights from in vitro and in vivo models. Cell Cycle *7*, 57–62.

Meredith, J. E., Fazeli, B., and Schwartz, M. A. (1993). The extracellular matrix as a cell survival factor. Mol. Biol. Cell *4*, 953–961.

Ning, S., Nemeth, J. A., Hanson, R. L., Forsythe, K., and Knox, S. J. (2008). Anti-integrin monoclonal antibody CNTO 95 enhances the therapeutic efficacy of fractionated radiation therapy in vivo. Mol. Cancer Ther. *7*, 1569–1578.

Schwartz, M. A. (1993). Spreading of human endothelial cells on fibronectin or vitronectin triggers elevation of intracellular free calcium. J. Cell Biol. *120*, 1003–1010.

Schwartz, M. A., McRoberts, K., Coyner, M., Andarawewa, K. L., Frierson, H. F., Jr., Sanders, J. M., Swenson, S., Markland, F., Conaway, M. R., and Theodorescu, D. (2008). Integrin agonists as adjuvants in chemotherapy for melanoma. Clin. Canc. Res. 14, 6193–6197.

Simpson, C. D., Anyiwe, K., and Schimmer, A. D. (2008). Anoikis resistance and tumor metastasis. Cancer Lett. *272*, 177–185.

Truong, T., Sun, G., Doorly, M., Wang, J.Y.J., and Schwartz, M. A. (2003). Modulation of DNA damage-induced apoptosis by cell adhesion is independently mediated by p53 and c-Abl. Proc. Natl. Acad. Sci. USA *100*, 10281–10286.

Wiesen, J., and Werb, Z. (2000). Proteinases, cell cycle regulation, and apoptosis during mammary gland involution (minireview). Reprod. Dev. *56*, 534–540.