CORRELATION OF RESISTANCE TO APOPTOSIS WITH TUMOR PROGRESSION AND METASTASIS IN ORAL CAVITY SQUAMOUS CELL CARCINOMA

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Abstract: Oral cavity squamous cell carcinoma (OSCC) is one of the leading causes of cancer deaths worldwide, most of these resulting from local-regional recurrence and metastases. Evasion of apoptosis is an important hallmark of cancer development and progression. Anoikis, or detachment-induced apoptosis, has been proposed as a protective mechanism which operates to prevent growth of displaced epithelial and endothelial cells in inappropriate environments. Loss of anoikis has been identified as one of the key steps in progression towards invasiveness and metastasis in epithelial tumour progression. The discovery of the critical pathways of intracellular signalling cascades to the development of adhesion-independent survival during tumourigenesis *in vivo*, would provide a powerful target for drug therapy against tumour invasion and metastasis.

Oral cavity squamous cell carcinoma (OSCC) is one of the leading causes of cancer deaths worldwide. Most of these deaths result from local-regional recurrence and metastases, despite improvements in surgery, radiotherapy, and conventional chemotherapy, leading to 5-year survival rates that remain at only 30–50%. Although nodal metastases and extension of the tumor beyond the lymph node capsule are considered the most predictive factors for regional recurrence and death, little is known about the biologic basis of tumor progression (Mandal et al., 2006). Evasion of apoptosis is an important hallmark of cancer development and progression, and several studies have shown that evasion of anoikis, or detachment-induced apoptosis, correlates with a more aggressive phenotype of carcinoma cells in OSCC (Kupferman et al., 2007).

Anoikis was originally defined by Frisch about a decade ago as a unique phenomenon reflecting apoptotic cell death consequential to insufficient cell-matrix interactions (Frisch & Francis, 1994). Anoikis can suppress expansion of oncogenically transformed cells by preventing proliferation at migrating locations, whereas migrating tumor cells that are resistant to anoikis induction can grow at inappropriate locations (Zhu et al., 2001; Ramachandra et al., 2002). Resistance to anoikis is thus emerging as a hallmark of metastatic cancer cells, especially because anchorage-independent growth of tumor cells is a classic characteristic of different types of human malignancies (Frisch & Screaton, 2001).

In a multicellular organism, cells do not exist in isolation; they associate with the neighbouring cells and the extracellular environment. The ECM (extracellular matrix) provides the physical scaffold on which the cells adhere and also provides cells with information required for proliferation, migration, differentiation and survival (Valentijn, Zouq & Gilmore, 2004).

The basement membrane (BM) is a specialized form of the ECM. In epithelial tissues it separates the epithelial cell population from the stroma and is a key player in both the maintenance of normal tissue architecture and in tumor development. The BM functions as a semipermeable barrier between the epithelial and stromal cells preventing the free passage from one side to another of certain biologically active molecules such as growth factors, representing at the same time a depot for storage of these compounds. Releasing the growth factors from the BM by the actions of certain extracellular proteases elicits specific mitogenic and survival responses. In pathological states such as inflammation or tumorigenesis the normal functioning of the ECM may be severely altered. For example, many primary tumors secrete various ECM components in abundance, thereby altering the cells’ immediate environment. Moreover, the extracellular proteases that are present in increased amounts in aggressively growing tumor masses (many

produced by recruited inflammatory cells) selectively degrade components of the BM, resulting in mobilization of sequestered growth factors (Jacks & Weinberg, 2002).
Disruption of substrate adhesion in normal epithelial cells rapidly induces programmed cell death. *In vivo*, anoikis would result from detachment of viable epithelial cells from the BM and could play an important protective role in preventing actively proliferating cells from re-attachment and growth in inappropriate environments (Bretland, Lawry & Sharrard, 2001).
Attachment to the ECM is mainly mediated by integrins. These are a family of heterodimeric transmembrane receptors composed of an alpha and a beta chain. In response to physiological stimuli, integrin signaling mediates cell differentiation, proliferation, homing, migration and survival. Because integrins lack kinase domains, they signal by associating in complexes with other mediators such as FAK, ILK, Src, Shc, Syk, and paxillin (Díaz-Montero & McIntyre, 2005). Signals propagated from cell–ECM adhesion complexes activate a number of well-characterized pathways, many of which have been suggested to play a role in the suppression of apoptosis (Valentijn, Zouq & Gilmore, 2004). The underlying mechanisms rendering tumor cells resistant to anoikis are not fully understood, but it has been postulated that it may comprise the stimulation of survival signals that are not extracellular matrix (ECM) contact dependent and inhibition of apoptotic pathways (Rennebeck, Martelli & Kyprianou, 2005).
Research over the past decade has elucidated the regulation of Akt kinase by upstream signaling events, mainly as a consequence of activation of the second messenger phospholipid kinase phosphatidylinositol 3-kinase (PI3K) and also established a role for Akt in promoting cell survival. (Brazil & Hemmings, 2001; Scheid & Woodgett, 2001, 2003). Critical to the understanding of the regulation of Akt in cells was the finding that Akt kinase activity is induced following PI3K activation in various growth factor receptor-mediated signaling cascades (Nicholson and Anderson, 2002).
The phosphatidylinositol-3 kinase (PI3K)/Akt signaling pathway has been shown to mediate tumor cell proliferation. Moreover, it seems to be up-regulated in a number of tumor types, including oral squamous cell carcinoma (Mills et al., 2001; Dong et al., 2001; Worsham et al., 2003). Nakayama and coworkers (2001) have shown that higher levels of Akt expression contribute to malignancy and antiapoptotic activity in squamous cell carcinoma of oral epithelium. The Akt pathway has also been shown to be important in mediating signals that lead to anchorage-independent survival, and inhibition of the PI3K/Akt has been shown in other cell types to lead to cell death by anoikis (Douma et al., 2004; Zhan et al., 2004). Li et al. (1989) showed that selection for resistance to anoikis leads to aggressive tumor growth and decreased animal survival in an orthotopic model of tongue cancer in nude mice. Given the association between anoikis resistance and the tumor progression of oral squamous cell carcinoma and the associations between anoikis resistance and the PI3K/Akt pathway, Mandal and coworkers (2006) evaluated the effects of a specific Akt inhibitor, KP372-1 on the inhibition of PI3K/Akt pathways biochemically and on cell proliferation, apoptosis, and anoikis in head and neck cancer cell lines. The authors came to the conclusion that activation of Akt appears to be a key step in the development of squamous cell carcinoma. An increase in Akt activity in head and neck squamous cell carcinoma (HNSCC) was also found in a large fraction of HNSCC-derived cell lines, irrespective of whether the EGF receptors were activated in these cells (Sriuranpong et al., 2003). Gupta and coworkers (2002) found a significant association between Akt activation and decreased local tumor control in a series of 38 patients with head and neck cancer. Amornphimoltham and coworkers (2004) recently showed that UCN-01, which inhibits the Akt pathway by inhibiting PDK1, displayed potent antiproliferative properties in squamous cell

carcinoma cell lines, leading to apoptosis. Thus, in HNSCC tumor xenograft models, treatment with UCN-01 for 5 consecutive days led to sustained inhibition of tumor growth, even after only one cycle of UCN-01 treatment. These effects were associated with a high incidence of apoptosis. Thus, these observations, taken together with the frequent occurrence of Akt activation squamous cell carcinoma cell lines, indicate that the Akt pathway could be a useful target for therapy.

The intracellular signaling mechanisms regulating survival and death are responsive to the interaction of transmembrane adhesion molecules with the ECM. The extracellular signals, cell surface receptors and signalling pathways involved in the regulation of epithelial cell survival have yet to be fully elucidated. Some of the molecular events that are involved in the inhibition of anoikis in tumor cells have been identified but the knowledge about the mechanisms by which these cells escape anoikis is far from complete.

REFERENCES

Amornphimoltham, P., Sriuranpong, V., Patel, V., Benavides, F., Conti, C.J., Sauk , J. et al., 2004. *Clin Cancer Res*, 10(12 Pt 1), 4029–37.

Bretland, A. J., Lawry, J. and Sharrard, R.M., 2001. *Cell Prolif.*, 34, 199–210.

Díaz-Montero, C. M. and McIntyre, B.W., 2005. *BMC Cancer*, 5, 39.

Dong, G., Chen, Z., Li, Z.Y., Yeh, N.T., Bancroft, C.C., Van Waes, C., 2001. *Cancer Res*, 61(15), 5911–8.

Frisch, S.M., Francis, H., 1994. *J Cell Biol,* 124, 619–26.

Douma, S., Van Laar, T., Zevenhoven, J., Meuwissen, R., Van Garderen, E., Peeper, D.S., 2004. *Nature,* 430, 1034–9.

Frisch, S.M., Screaton, R.A., 2001. *Curr Opin Cell Biol*,13: 555–62.

Gupta, A.K., McKenna, W.G., Weber, C.N., Feldman, M.D., Goldsmith, J.D., Mick, R.. et al., 2002, *Clin Cancer Res*, 8(3), 885–92.

Jacks , T. and Weinberg, R.A., 2002. *Cell*, 111, 923–925.

Kupferman, M.E., Patel,V., Sriuranpong, V., Amornphimoltham, P., Jasser, S.A., Mandal, M. et al., 2007. *Oral Oncol*., 43(5), 440-454.

Li, L., Price, J.E., Fan, D., Zhang, R.D., Bucana, .C.D., Fidler I.J. 1989, *J Natl Cancer Inst,* 81(18), 1406–12.

Mandal, M., Younes, M., Swan, E.A., Jasser, S.A., Doan, D., Yigtbasi, O.et al., 2006. *Oral Oncol*., 42(4), 430–439.

Mills, G.B., Lu, Y., Fang, X., Wang, H., Eder, A., Mao, M. et al., 2001. *Semin Oncol*, 28(5 Suppl 16), 125–41.

Nakayama, H., Ikebe, T., Beppu, M., Shirasuna, K., 2001. *Cancer,* 92(12), 3037–44.

Nicholson, K.M. and Anderson, N.G., 2002. *Cell. Signal*., 14, 381–395.

Ramachandra, M., Atencio, I., Rahman, A., et al., 2002. *Cancer Res*, 62, 6045–51.

Rennebeck, G., Martelli, M. and Kyprianou, N., 2005. *Cancer Res*, 65(24), 11230-5.

Scheid, M.P. and Woodgett, J.R., 2001. *Nat. Rev. Mol. Cell Biol*., 2, 760–768.

Scheid, M.P. and Woodgett, J.R., 2003. *FEBS Lett*., 546, 108–112.

Sriuranpong, V., Park, J.I., Amornphimoltham, P., Patel, V., Nelkin, B.D., Gutkind, J.S., 2003, *Cancer Res*, 63(11), 948–56.

Valentijn, A.J., Zouq, N. and Gilmore, A.P., 2004. *Biochemical Society Transactions*, 32: 421-425.

Worsham, M.J., Pals, G., Schouten, J.P., Van Spaendonk, R.M., Concus, A., Carey, T.E. et al., 2003. *Arch Otolaryngol Head Neck Surg*, 129(7), 702–8.

Zhan, M., Zhao, H. et al. 2004, *Histol Histopathol*, 19(3), 973–83.

Zhu, Z., Sanchez-Sweatman, O., Huang, X,, et al., 2001. *Cancer Res*, 61: 1707–16.

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