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Regulation of mammary epithelial cell phenotypes by the helix-loop-helix protein, Id-1

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Abstract

Mammary epithelial cells undergo cycles of proliferation, invasion, differentiation and apoptotic cell-death throughout adult life. The molecular mechanisms that regulate these complex and co-ordinated developmental programs are poorly understood. We have identified Id-1 protein, a negative regulator of basic helix-loop-helix transcription factors, as a critical regulator of these normal mammary epithelial cell phenotypes. We also found that Id-1 is an important regulator of the aggressive and invasive phenotype, as well as mediator of the effects of sex steroid hormones, in human breast cancer cells.

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Introduction

The mammary gland is one of the few organs that undergo striking changes throughout adult life. During each menstrual cycle, and especially during pregnancy, lactation and involution, mammary epithelial cells go through cycles of proliferation, invasion, differentiation and apoptotic cell death. The mechanisms that regulate these complex and co-ordinated developmental programmes are only poorly understood. Some of the downstream genes that are regulated during different stages of mammary development have been identified. However, very little is known about the transcriptional regulators that control the expression of these developmental stage-specific genes.

The *Id-1* gene as regulator

We have identified the *Id-1* gene as a critical regulator of mammary epithelial cell growth, differentiation and invasion. Id proteins are a small family of short proteins (13-20 kDa) that function as dominant negative regulators of basic helix-loop-helix (bHLH) transcription factors (Benezra *et al.* 1990). bHLH proteins activate transcription as homo- or heterodimers. The HLH domains cause dimerization, bringing the basic domains together, which in turn bind DNA at the promoters of target genes. This class of proteins has been found to activate transcription of cell- and tissue-specific genes that direct terminal differentiation and cell-lineage commitment. Id proteins contain HLH domains, but lack basic domains. As a result, heterodimers between an Id protein and a bHLH protein cannot bind DNA and are non-functional.

We found that, during normal breast development and remodelling in mouse, Id-1 expression is inversely correlated with that of β-casein, a marker of breast epithelial cell functional differentiation. Id-1 mRNA is highly expressed during the initial stages of development, declines to undetectable levels when the mammary gland proceeds towards full differentiation, and is again highly expressed at the beginning of involution. Id-1 expression also declines rapidly when SCp2 mouse mammary epithelial cells are induced to differentiate (Desprez *et al.* 1995). Ectopic expression of Id-1 in these cells confers an increase in proliferation, migration and invasiveness. When cultured in the presence of lactogenic hormones and a reconstituted basement membrane, Id-1-transfected cells form loose alveolar structures from which cells eventually dissociate, invade the basement membrane and then resume proliferation (Desprez *et al.* 1998). In contrast, control cells remain as stable alveolar structures. Moreover, we have shown that Id-1 expression induces the expression of a novel metalloproteinase of 120 kDa, indicating a potential role for Id-1 in the progression of some breast cancers towards aggressive malignant stages.

In human breast cancer cells grown under serum-free conditions, Id-1 mRNA and protein (in addition to its induced 120 kDa metalloproteinase) are found to be

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constitutively expressed in the most aggressive and invasive cells in comparison with more differentiated breast cancer cell lines (Desprez *et al.* 1998). We also found that Id-1 gene expression is under the control of oestrogen and progesterone. Moreover, Id-1 can mediate the effect of these two hormones on the proliferation of breast cancer cells. These findings provide molecular mechanisms for some previously well known, yet poorly understood actions of these sex steroid hormones in the treatment of advanced breast cancer (Lin CQ, Campisi J & Desprez P-Y unpublished observations). Thus Id-1 seems to be a critical regulator of mammary epithelial cell phenotype repertoire, mediating growth, differentiation and invasion.

We recently examined the involvement of Id-1 in the regulation of the massive programmed cell death that occurs during involution of the mammary gland. Three earlier publications had reported a role for Id proteins in the regulation of apoptosis in non-epithelial cells. Under serum-free conditions, Id-3 induced apoptosis in fibroblasts (Norton & Atherton 1998). Id-2 was found to induce apoptosis in both myeloid precursors and osteosarcoma cells by an HLH-independent mechanism, and the death-promoting function was localised to the N-terminus of Id-2 (Florio *et al.* 1998). Finally, the suppression of adenovirus E1A-induced apoptosis by mutated p53 was overcome by co-expression of Id proteins in rat embryo fibroblasts (Nakajima *et al.* 1998).

We found that ectopic Id-1 expression, by transfection with an Id-1 expression vector, triggered proliferation in SCp2 mammary epithelial cells when the cells were cultured at subconfluence in serum-free medium. However, the same cells underwent apoptosis when confluent in serum-free medium. The SCp2 cells transfected with an insertless vector or anti-sense Id-1 cDNA did not undergo apoptosis under these conditions. These findings provide a cell culture model that mimics mammary gland development. During certain stages of this development, the ductal and lobulo-alveolar epithelial cells proliferate and invade the surrounding stroma. We found that Id-1 is highly expressed under these conditions. At the beginning of involution, Id-1 is reexpressed when mammary epithelial cells, packed inside the fully differentiated alveoli, undergo apoptosis. We are currently identifying the mechanisms by which Id-1 protein promotes either growth or apoptosis, depending on the cell context and developmental stage.

Taken together, these data indicate that Id-1 is a key regulator of mammary epithelial cell proliferation, invasion, differentiation and death during different stages of mammary gland development, and in the malignant progression of breast cancer.

References

Benezra R, Davis RL, Lockshon D, Turner DL & Weintraub H 1990 The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. *Cell* **61** 49-59.

Desprez PY, Hara E, Bissell MJ & Campisi J 1995 Suppression of mammary epithelial cell differentiation by the helix-loop-helix protein, Id-1. *Molecular and Cellular Biology* **15** 3398-3404.

Desprez PY, Lin CQ, Thomasset N, Sympson CJ, Bissell MJ & Campisi J 1998 A novel pathway for mammary epithelial cell invasion induced by the helix-loop-helix protein Id-1. *Molecular and Cellular Biology* **18** 4577-4588.

Florio M, Hernandez MC, Yang H, Shu HK, Cleveland JL & Israel MA 1998 Id2 promotes apoptosis by a novel mechanism independent of dimerization to basic helix-loop-helix factors. *Molecular and Cellular Biology* **18** 5435-5444.

Nakajima T, Yageta M, Shiotsu K, Morita K, Suzuki M, Tomooka Y & Oda K 1998 Suppression of adenovirus E1A-induced apoptosis by mutated p53 is overcome by coexpression with Id proteins. *Proceedings of the National Academy of Sciences of the USA* **95** 10590-10595.

Norton JD & Atherton GT 1998 Coupling of cell growth control and apoptosis functions of Id proteins. *Molecular and Cellular Biology* **18** 2371-2381.