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The Stat3 paradox: A killer and an oncogene

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ABSTRACT

Stat proteins regulate many aspects of mammary gland development, including the profound changes that occur during pregnancy, lactation and involution. Stat3 induces transcriptional activation of genes involved in the inflammatory response, and in seemingly contradictory cellular events such as apoptosis, differentiation and stem cell maintenance. While Stat3 signalling during mammary gland involution induces epithelial cell death, aberrant Stat3 activation is widely implicated in breast tumourigenesis. Specific cytokines may initiate either a Stat3-driven proliferative or death response depending on the cell-type and cell-context specific availability of particular combinations of signals and receptors. The paradoxical functions of Stat3 may also be due to the degree and extent of activation in different circumstances, in addition to paracrine signalling between mammary epithelial cells and the surrounding microenvironment. Deciphering the enigmatic nature of Stat3 in the mammary gland may benefit future therapeutic strategies for inducing cell death in breast tumours.

1. Introduction

Transcription factors of the Signal Transducers and Activators of Transcription (Stat) family act downstream of cytokines and growth factors to mediate cell communication and induce key biological responses (Levy and Darnell, 2002). Seven mammalian Stat proteins have been described that are involved in diverse processes, from inflammation, differentiation and stem cell maintenance to cell proliferation, death and malignant transformation. Cytokine binding to cell surface receptors results in the activation of the Janus kinase (Jak) family of kinases, which recruit and phosphorylate specific latent Stats in the cytoplasm depending on the particular receptor-Jak combination (Darnell et al., 1994). Activation by tyrosine phosphorylation leads to Stat dimerisation and translocation into the nucleus where they regulate the transcription of target genes specific to each Stat (Darnell et al., 1994). Various external stimuli trigger a variety of receptors that activate different, cell-type specific Stat proteins and subsequent gene transcriptional programs, thus allowing a degree of context specificity in cellular response. Many of the Stat family members are expressed in the mammary gland, mediating its development and cellular response during pregnancy, lactation and involution, and are widely implicated in breast tumourigenesis (reviewed elsewhere in this issue (Haricharan and Li, 2013)).

Stat3 was originally discovered as a transcription factor activated during inflammatory cytokine signalling, and was initially named acute phase response factor (APRF) due to its regulation of genes involved in the acute phase response (Akira et al., 1994). Stat3 is constitutively expressed in a wide range of tissues and regulates the transcriptional activation of genes not only involved in the inflammatory response, but also in seemingly contradictory cellular events such as apoptosis, differentiation and stem cell maintenance (Dauer et al., 2005). Unlike other Stat proteins gene targeting of Stat3 in mice is lethal during early embryogenesis and in embryonic stem cells, with homozygous Stat3-null embryos degenerating rapidly between 6.5 and 7.5 days (Takeda et al., 1997; Raz et al., 1999).

Stat3 is activated in response to distinct signals, including certain growth factors, interferons (IFNs) and the gp130 family of cytokines, such as interleukin (IL)-6, oncostatin M (OSM) and leukemia inhibitory factor (LIF) (Schindler et al., 2007). Growth factor receptors possessing intrinsic tyrosine kinase activity, such as epidermal growth factor receptor (EGFR), can result in Stat3 activation, in addition to a number of oncogenes, including c-Src, c-Abl and ErbB2. Indeed, Stat3 is reported to be constitutively phosphorylated in many cancers and appears to promote tumour survival and progression (Turkson and Jove, 2000; Yu and Jove, 2004). Stat3 also mediates cell survival in physiological non-tumourigenic settings, such as acting as a potent pro-survival factor in T-cells (Takeda et al., 1998) and as a cytoprotective mediator during ischemia/reperfusion (I/R) injury in primary rat myocytes (Barry et al., 2009). Intriguingly, the biological consequences of Stat3 activation appear to be highly cell-type and cell-context specific. For example, while Stat3 is considered an oncogene and drives the proliferation and survival of many breast tumour types (Levy and Inghirami, 2006), Stat3 activation during mammary gland involution induces mammary epithelial cell death (Chapman et al., 1999). This

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apparent discrepancy between its physiological and pathological roles in the mammary gland is a focus of intensive interest. This article will highlight the paradoxical nature of Stat3 signalling in the mammary gland by describing its function in mediating cell death during involution and contrasting this with the role of Stat3 in breast tumourigenesis.

2. Stat3 and mammary gland involution

Prior to being implicated as an oncogene, one of the first biological roles ascribed to cytokine-induced Stat3 signalling was cell death. In this context, IL-6 induced death of myeloid leukaemia M1 cells was demonstrated to be dependent on Stat3 activation (Minami et al., 1996; Nakajima et al., 1996; Yamanaka et al., 1996). Stat3 is also reported to be crucial for the cell death of T cells after MHC-1 ligation (Skov et al., 1998) and of neuronal cells due to beta-amyloid accumulation (Wan et al., 2010), in addition to being recently implicated in programmed necrosis (necroptosis) (Shulga and Pastorino, 2012). However, the physiological setting in which involvement of Stat3 in cell death has been interrogated most comprehensively is the mammary gland.

The mammary gland undergoes three stages of development: in the embryo, during puberty and in adulthood. Several hormones and growth factors regulate the early processes of placode formation and ductal branch formation, in addition to alveologenesis during pregnancy, whereby alveolar progenitor cells are stimulated to proliferate and expand the glandular tissue (Watson and Khaled, 2008). The resulting secretory alveolar cells are responsible for milk production to nurture the young until they are weaned, which subsequently leads to cell death and widespread tissue remodelling that returns the gland into a ''pre-pregnancy'' state. This post-lactational regression, termed involution, occurs in two phases consisting of two different modalities of cell death. Furthermore, involution is reversible during its first phase, such that re-introduction of the pups can re-initiate lactation (Lund et al., 1996). As mentioned, Stat signalling is known to carefully orchestrate mammary gland development, and Stat3 is specifically upregulated upon initiation of the involution process (Liu et al., 1996; Philp et al., 1996). Moreover, involution appears to be dependent on intact Stat3 signalling as mice with a mammary epithelium-specific knockout of Stat3 display a severe delay in post-lactational regression of the gland (Chapman et al., 1999; Humphreys et al., 2002). These studies were pivotal in ascribing a role for Stat3 as an initiator of cell death *in vivo* and over 60 studies have since investigated Stat3-mediated cell death during mammary gland involution.

A variety of cytokines are crucial for inducing both Stat3 and NF-κB signalling during post-lactational regression of the mammary gland, including LIF, which was the first cytokine to be identified as a physiological inducer of Stat3 in this context. Studies in LIF-deficient mice revealed that the activation of Stat3 post-weaning depends on LIF (Kritikou et al., 2003), while implantation of LIF-containing pellets into the lactating mammary gland resulted in epithelial cell death alongside Stat3 induction (Schere-Levy et al., 2003). During the second phase of involution OSM takes precedence and is the key cytokine that regulates Stat3 activation via OSM-receptor signalling (Tiffen et al., 2008) (Fig. 1). Additionally, transforming growth factor beta3 (TGF-β3) has also been implicated in regulating apoptosis during mammary gland regression (Nguyen and Pollard, 2000).

Whilst the mechanism of Stat3-mediated cell death in the mammary gland has been studied for over 15 years, it was only recently revealed that LIF-induced cell death during the first phase of involution is due to a lysosomal-mediated programme of cell death, and not classical apoptosis (Kreuzaler et al., 2011). Cell death during the first 2 days of post-lactational regression was demonstrated to be independent of executioner caspases, as involution appeared unperturbed in caspase 3/6-knockout mice and upon overexpression of the caspase inhibitor, p35 (Kreuzaler et al., 2011). Additionally, transmission electron microscopy revealed that dead cells shed during the first phase of involution possessed morphological features that were atypical for apoptosis, such as cell swelling and a complete lack of membrane blebbing. Dead cells were also found to stain TUNEL-negative until the second phase of involution, during which a strict, temporal activation of caspase 7 could be detected. Further investigation showed that the first phase of involution is marked by a downregulation of the lysosomal membrane protein Lamp2 and initiation of lysosomal cysteine cathepsin expression. Whilst lysosomes isolated from lactating tissue did not appear to leak any protease content, time-dependent leakage of cathepsins could be detected after 24 h of involution, with protease activity detected both in the lysosomes and cytosol of cells from the involuting mammary gland. Concurrently, expression levels of the endogenous cathepsin inhibitor Spi2A is abruptly reduced during involution, allowing for amplified cathepsin activity in the cytosol, which is known to initiate various forms of cell death (Kirkegaard and Jaattela, 2009). Both Spi2A and cathepsin expression appeared to be differentially regulated by Stat3, as Stat3-knockout glands showed prolonged expression of Spi2A without an induction of cysteine cathepsin expression during involution (Kreuzaler et al., 2011). In support, OSM induced Stat3 phosphorylation in a murine mammary epithelial cell line was demonstrated to result in upregulated cathepsin expression and subsequent lysosomal destabilisation, leading to caspase-independent cell death. Stat3 is thus a regulator of an alternative physiological form of lysosomal-mediated programmed cell death, which is initiated in the first phase of post-lactational mammary gland regression (Kreuzaler et al., 2011).

LIF-induced Stat3 activation in the first, reversible phase of involution leads to the upregulation of the OSM-receptor by day 2 of involution. Accordingly, in the second phase of mammary gland regression, Stat3 activation by OSM is observed while LIF-levels in the gland decline (Tiffen et al., 2008). This switch to the second phase causes dramatic remodelling of the architecture of the mammary gland, which stays intact during the first phase of involution. Extracellular matrix (ECM) degradation by matrix metalloproteinases (MMPs) is crucial for remodelling, and expression of tissue inhibitors of metalloproteinases (TIMPs) that block MMP activity in the first phase is diminished at this point (Green and Lund, 2005). The degradation of ECM is likely to cause cell detachment from the basement membrane, which may in turn contribute to the occurring cell death by anoikis (detachment-induced cell death). Furthermore, TUNEL-positivity and upregulation of caspase 3, 6 and 7 cleavage (Kreuzaler et al., 2011) point towards an increase in apoptotic cell death during the second phase of involution. Hence, within the same organ, Stat3 temporally regulates lysosomal and subsequent classical programmed cell death activated by LIF- and OSM-mediated signalling respectively.

Next to its role in initiating cell death during involution, Stat3 was also shown to upregulate genes associated with the acute phase response and inflammation (Clarkson et al., 2004; Stein et al., 2004). In the first phase of involution this includes genes related to innate immunity, while the second phase sees an upregulation of wound healing genes (Hughes et al., 2012). The role of Stat3 in the acute phase response has also been described in response to infection and tissue injury, and it is known to contribute to the inflammatory microenvironment of Stat3-addicted cancers (Pensa et al., 2009). Stat3 activation therefore induces the inflammatory response in the context of cell death during involution, in addition to promoting cell survival in tumourigenesis, which will be further described below.

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