

Checkpoints and Cell Cycle Control

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Cancer results from genetic errors or mutations that subvert the normal controls governing cell proliferation and differentiation, yet it is commonly treated with radiation and genotoxic drugs that either damage or inhibit replication of the genetic material, DNA. Such treatments are however imperfect and can have serious adverse side effects. Our research focuses on understanding the molecular mechanisms that enable tumour cells to respond to and survive exposure to genotoxic therapies. By understanding these mechanisms, and how they interact with those that control normal cell growth and division, we hope to find ways to make anti-cancer therapies more effective or to mitigate their unwanted side effects.

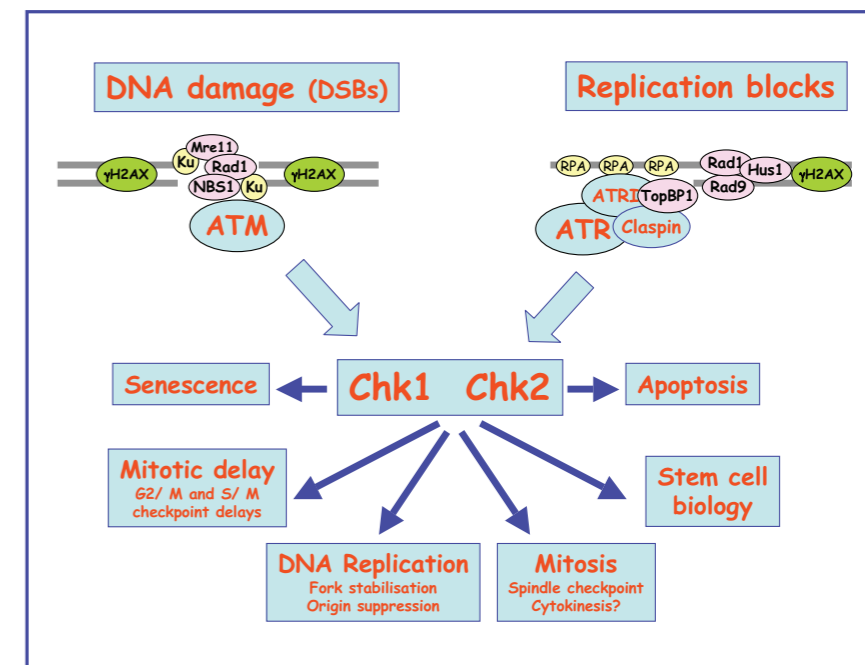
Checkpoints are molecular alarm mechanisms that signal the presence of damaged or unreplicated DNA and trigger cellular responses that minimise the risk of lethal or permanent genetic damage. Checkpoint signals are relayed *via* complex signal transduction pathways to activate two protein kinases, Chk1 and Chk2, which then orchestrate appropriate cellular responses. Checkpoints control a remarkable diversity of cellular processes, ranging from cell cycle progression, DNA replication and chromosome segregation to cell senescence and survival (Fig. 1). Checkpoints are activated acutely by cancer chemo- and radiotherapy, when they are likely to favour tumour cell survival, however incipient cancer cells are also subject to spontaneous genotoxic stress due to oncogene activation early during their evolution. In this situation, checkpoints may suppress tumourigenesis by promoting cancer cell senescence or apoptosis. The role of checkpoints and DNA damage signalling in the evolution and therapy of cancer is therefore complex and context-dependent.

In recent years, it has become apparent that the Chk1 kinase is the key effector of most of the cell cycle checkpoint responses in tumour cells. Thus, Chk1-deficient lymphoma cells cannot arrest in G2/M in response to ionising radiation, even at very high doses that induce massive amounts of DNA damage (Zachos *et al.*, EMBO J. 2003; 22: 713). Chk1 is also essential for the

replication checkpoint functions that stabilise stalled replication forks, inhibit replication origin firing and delay the onset of mitosis (the S/M checkpoint) when DNA synthesis is blocked (Fig. 1). Failure of this latter checkpoint results in Chk1-deficient tumour cells entering mitosis prematurely with unreplicated DNA when replication is blocked, resulting in mitotic catastrophe and cell death (Zachos *et al.*, Mol. Cell. Biol. 2005; 25: 563; Zachos *et al.*, Mol. Cell. Biol. 2007; 12: 247). In comparison, genetic inactivation of Chk2 in lymphoma cells modestly impairs DNA damage-induced G2/M arrest but has little, if any, consequence for replication checkpoint proficiency (Rainey *et al.*, Oncogene 2008; 27: 896). A major objective now is to understand how Chk1 controls such a multitude of diverse processes, to identify the relevant downstream substrates and determine how its biochemical activity is directed to appropriate targets in response to specific signals.

Other studies have provided evidence that Chk1 is an important factor in determining whether tumour cells survive exposure to radiation and anti-cancer drugs. Thus, Chk1-deficient lymphoma cells are extremely sensitive to ionising radiation (Zachos *et al.*, EMBO J. 2003; 22: 713) and replication-inhibiting anti-metabolite drugs such as 5-fluorouracil (Robinson *et al.*, Oncogene 2006; 25: 5359). Importantly, newly-developed pharmacological Chk1 inhibitors, some of which

Figure 1
Chk1 and Chk2 functions in vertebrate cells. DNA double strand breaks (DSBs) or single-stranded DNA tracts arising from stalled replication forks are recognised by sensor molecules leading to the activation of the ATM/ATR and Chk1/Chk2 protein kinase cascades and checkpoint activation. In the case of DSBs, nucleolytic DNA strand resection, which is dependent on ATM activity, is required for activation of ATR-Chk1. See text for details.



are currently in clinical trials (Smith *et al.*, Adv. Cancer Res. 2010; 189: 73), can reproduce these effects and enhance tumour cell killing by radiation and conventional chemotherapy agents (Fig. 2).

Remarkably, genetic inactivation of Chk1 can also suppress the formation of skin tumours induced by chemical carcinogens *in vivo*, indicating that Chk1 inhibition can have an inherently anti-cancer effect, even in the absence of concurrent genotoxic treatment. This effect is likely to be mediated at the level of keratinocyte stem cells, a principal target of chemical carcinogens, which we believe require Chk1 for their survival or proliferation. Understanding how Chk1 inhibition affects the initiation and progression of

squamous and melanoma skin tumours is therefore an important future objective.

Chk1 emerges therefore as a 'master regulator' of multiple checkpoint responses and a crucial arbiter of survival in tumour cells exposed to genotoxic stress. Pharmacological inhibition of Chk1 therefore provides a rational strategy for enhancing the efficacy of existing genotoxic anti-cancer therapies. In future, it will be important to extend these studies to clinically important agents with different mechanisms of action and to understand how checkpoint suppression can lead to tumour cell killing at the molecular level.

Publications listed on page 77

Figure 2
Pharmacological inhibition of Chk1 enhances tumour cell killing by radiation and conventional anti-cancer agents that act both by inducing DSBs and by stalling DNA replication forks. The consequences of Chk1 inhibition include; escalation of damage, formation of more lethal lesions, inhibition of DNA repair leading to increased tumour cell death through apoptosis or novel mechanisms such as mitotic catastrophe.

