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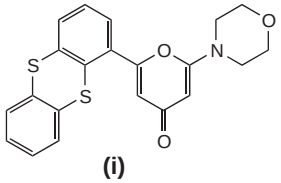
monitor

MOLECULES

Sensitization to radio- and chemo-therapy by a novel inhibitor of ATM kinase

Ataxia-telangiectasia mutated (ATM) kinase is a serine/threonine kinase known to have an important role in the maintenance of genomic integrity via signalling to cell cycle and DNA repair pathways, involving phosphorylation of targets such as p53, CHK2, NBS1 and BRCA1 [1]. The hypothesis that ATM inhibition would sensitize cancer cells to radio- and chemo-therapy has previously been tested using the relatively non-specific phosphatidylinositol 3′-kinase (PI3K)-related kinase family inhibitors wortmannin and caffeine, which are known to have spectra of kinase inhibitory effects that include ATM [2].

The screening of a combinatorial compound library based on the non-specific PI3K and DNA-dependent protein kinase inhibitor LY294002 led to the identification of KU55933 (**i**), a novel, specific and potent small molecule inhibitor of ATM [3]. In addition to inhibiting ATM with an IC_{50} of 13 nM and a K_i of 2.2 nM, KU55933 also showed specificity for inhibition of ATM with respect to a panel of related kinases. KU55933 also sensitized HeLa cells to a range of radiation doses with a sensitizer enhancement ratio of 2.6 at 2.0 Gy (KU55933 concentration of 10 μM). Cellular chemosensitization to DNA double-strand break-inducing agents, such as etoposide, doxorubicin and camptothecin, by KU55933 was also demonstrated. The identification of KU55933 provides a relatively specific molecular tool to study the cellular biochemistry of ATM, and a starting point for the development of more potent and pharmaceutically acceptable ATM inhibitors with future potential for clinical evaluation.



(i)

1 Shiloh, Y. *et al.* (2003) ATM and related protein kinases: safeguarding genome integrity. *Nat. Rev. Cancer* 3, 155–168
2 Sarkaria, J.N. *et al.* (1998) Inhibition of phosphoinositide 3-kinase related kinases by the radiosensitizing agent wortmannin. *Cancer Res.* 58, 4375–4382
3 Hickson, I. *et al.* (2004) Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. *Cancer Res.* 64, 9152–9159

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SIGNALLING

Forever young

Ageing diminishes the regenerative capacity of tissues. Previous studies have shown that the activation of the Notch signalling pathway is essential for the activation, proliferation and myogenic lineage progression of satellite cells necessary for muscle regeneration. In old muscles, the activation of this particular pathway fails. However, aged muscles successfully regenerate when grafted in young recipient, but young muscles display impaired regeneration when grafted in old recipient. These observations led to the hypothesis that there are systemic factors that support the regeneration of tissues in young animals and/or inhibit regeneration in old animals.

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To examine this hypothesis, Conboy and co-workers set up an experimental system in which - in contrast to transplantation-regenerating tissues in aged mice could be exposed only to the circulating factors of young mice, and vice versa. The authors established parabiotic pairings between young (2–3 months) and old (19–26 months) animals (heterochronic parabioses), and parabiotic pairings between two young and two old animals (isochronic parabioses) as controls. They examined the efficacy of muscle regeneration after injury in young and aged mice in heterochronic and isochronic pairings.

Five days after injury, muscles in young mice in isochronic and in heterochronic parabioses had regenerated. In contrast, injured aged muscle from isochronic parabioses regenerated poorly, but parabioses with young mice significantly enhanced the regeneration of muscles in old partners. Further experiments led to the conclusion that heterochronic parabiosis not only enhances the proliferative response of the aged progenitor cells, it also restores the key molecular signalling in these cells that is necessary for muscle regeneration.

Next, the authors determined whether the rejuvenating effects observed *in vivo* could be replicated *in vitro*. Satellite cells, cultured either alone or together with myofibre explants, were prepared from young and old mice and cultured in the presence of young or old mouse serum. The data showed that, as observed *in vivo*, components in young serum alone are capable of reversing the decline of aged muscles. In addition, it seemed that factors in old serum inhibit satellite cells activation and muscle repair. Similar data were collected with aged hepatocytes *in vivo* and *in vitro*.

These data show that a young systemic environment can restore a younger profile of molecular signalling pathways critical to the activation of tissue-specific cells. The next step will be to identify the factors able to reverse the regenerative potential of tissue-specific progenitor cells.

2 Conboy, I.M. *et al.* (2005) Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 433, 760–764

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Flexible alternative to rifampicin

Bacterial RNA polymerase (RNAP) is the target for rifampicin (Rif) and other low-molecular-weight inhibitors. Rif is used clinically in combination therapy to treat mycobacterial infections such as tuberculosis, but Rif-resistance is an increasing problem. Most Rif-resistant (Rif^R) mutants harbor amino acid substitutions in the part of RNAP that are located near or within

the binding pocket for Rif. This leads to reduced affinity of Rif to RNAP with reduced or absent inhibitory activity on bacterial RNA synthesis.

Campbell *et al.* [3], studied a chemically unrelated RNAP inhibitor, sorangicin A (Sor), isolated from a myxobacterium. Analysis of the activity of Rif and Sor on a number of RNAP mutations suggested that Rif and Sor binding sites overlap, but that there are differences in the interactions because only 5/11 substitutions lead to cross-resistance. Analysis of Sor-RNAP crystals showed that Sor interacts with RNAP in a nearly identical way as Rif, and biochemical analysis confirmed that both antibiotics terminate transcription in the same way. Structural analysis of resistance mutants indicated that Rif is very sensitive to changes in the shape of the binding pocket, whereas Sor is not, suggesting that Sor has a higher degree of conformational flexibility than Rif. Molecular dynamics simulations suggested that Sor has a much greater flexibility than Rif even under constraining conditions mimicking binding to RNAP.

In conclusion, this study shows by structural, functional and genetic analysis that sorangicin A is a much more flexible RNAP inhibitor than rifampicin making it less sensitive to RNAP mutations, leading to resistance. This could be instrumental in future rational design of new antibiotics to be used in treatment of tuberculosis and other bacterial infections.

3 Campbell, E.A. *et al.* (2005) Structural, functional, and genetic analysis of sorangicin inhibition of bacterial RNA polymerase. *EMBO J.* 24, 674–682

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MICROBIOLOGY

Streptococcus pyogenes can use fibrinogen to prevent opsonization

The M protein of *Streptococcus pyogenes* is an essential virulence factor that inhibits complement deposition and thereby helps bacteria avoid phagocytosis. Of the >80 M protein serotypes, many, such as M22, can bind human C4b-binding protein (C4BP), an inhibitor of the classical pathway of complement activation, which could explain how they inhibit complement deposition. However, other M proteins, such as M5, bind fibrinogen but do not bind C4BP. The fibrinogen-binding region of M5 has been localized to several 'B' repeats in the central portion of the protein. To determine whether the ability to bind fibrinogen can inhibit complement deposition, Carlsson *et al.* studied a mutant lacking the B repeats (ΔB) [1].

The M5 ΔB mutant protein was expressed on the bacterial surface and was still recognized by antibodies directed against N- and C-terminal domains. In a standard phagocytic assay, the ΔB mutation abolished the ability of the bacteria to replicate in nonimmune human blood whereas wild type and revertant strains exhibited doubling times of 19–20 minutes. Incubation of a mutant lacking the entire M5 protein (ΔM) or the ΔB mutant in nonimmune human plasma resulted in similar levels of complement deposition which occurred via the classical pathway. In contrast, there was ~ninefold less complement deposition on the wild-type M5 strain.

When this experiment was repeated using nonimmune serum, equivalent amounts of complement were deposited on the wild-type, ΔM, and ΔB strains. Addition of physiologic concentrations of fibrinogen to serum caused a substantial reduction of complement deposition on the M5 strain, whereas the ΔM and ΔB mutants were not affected. Further analysis suggested that binding of fibrinogen by M5 prevented the formation of C3 convertase rather than accelerating its degradation. These studies suggest that M proteins employ two distinct pathways, binding of C4BP or fibrinogen, to inhibit deposition of complement on the bacterial surface and raise the possibility that fibrinogen could have previously unrecognized anti-inflammatory properties in the human host.

1 Carlsson, F. *et al.* (2005) Human fibrinogen bound to *Streptococcus pyogenes* M protein inhibits complement deposition via the classical pathway. *Mol. Microbiol.* doi: 10.1111/j.1365-2958.2005.04527.x (E-pub. ahead of print; http://www.blackwell-synergy.com/rd.asp?code=MMI&goto=journal)

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