Modulation of p53 during bacterial infections

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Abstract | In recent years, numerous bacterial pathogens have been shown to inactivate the major tumour suppressor p53 during infection. This inactivation impedes the protective response of the host cell to the genotoxicity that often results from bacterial infection. Moreover, a new aspect of the antibacterial activity of p53 that has recently come to light — downregulation of host cell metabolism to interfere with intracellular bacterial replication — has further highlighted the crucial role of p53 in host–pathogen interactions, as host cell metabolism is relevant for all intracellular bacteria, as well as other pathogens that replicate inside host cells and use host metabolites. In this Progress article, we summarize recent work that has advanced our knowledge of the interaction between pathogenic bacteria and p53, and we discuss the known and expected outcomes of this interaction for pathogenesis.

The predominant role of the tumour suppressor p53 in regulating genomic stability and the DNA damage response makes it indispensable for maintaining cell integrity, and for this reason p53 has been called a "guardian of the genome" (REF. 1). In more than half of all human cancers, the gene that encodes p53 (*TP53*) is inactivated or carries missense mutations that result in the loss of its tumour suppressor function. In the past decade, reactivation of inactive p53 and recovery of its signalling pathways have constituted promising strategies for cancer therapies[2,3], and new insights into metabolic transformation in cancer cells led to the discovery of the regulatory role that p53 has in cellular metabolism[4]. Several metabolic changes are typically found in tumours, including enhanced rates of glucose uptake and high glycolytic flux under normal aerobic conditions, commonly referred to as the Warburg effect[5]. Our increasing understanding of these processes has led to the concept that these metabolic changes are the cause rather than the consequence of tumour formation[6,7].

The functions of p53 as a tumour suppressor are diverse, and the most well known of these include control of genomic stability, regulation of cell cycle arrest, induction of apoptosis and cellular senescence[8,9] (FIG. 1). As a protein with long-lasting activity, even at extremely low concentrations, the stability of p53 is subject to strict regulation, mainly through an autoregulatory negative feedback loop that it forms with the E3 ubiquitin ligase human double minute 2 (HDM2; also known as MDM2). HDM2 catalyses the formation of ubiquitin chains on p53 and thus mediates the constant export of p53 from the nucleus and its subsequent proteasomal degradation. In addition, a complex network of post-translational modifications involving phosphorylation, acetylation, ubiquitylation and sumoylation regulates the activation of p53 (REF. 8). Following induction of cellular stress, such as the DNA damage caused by many bacterial infections, the expression of oncogenes or oxidative stress, p53 is rapidly stabilized. Depending on the severity of DNA damage, p53 either initiates signalling pathways that lead to temporary cell cycle arrest and cellular repair or triggers the destruction of the damaged cell.

Upon detection of DNA double-strand breaks (DSBs), the protein kinases ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR)[10] and DNA-dependent protein kinase (DNA-PK) are activated and phosphorylate p53 and HDM2, as well as several other proteins involved in the DNA damage response (including the DSB marker histone H2AX, HDMA (or HDMX), p53-binding protein 1 (53BP1), checkpoint kinase 1 (CHK1) and CHK2 (REFS 11,12)). Nuclear foci with phosphorylated H2AX (known as γH2AX) promote repair of the cognate DSBs by attracting DNA repair proteins. To facilitate these repair processes, p53 arrests the cell cycle at the G1 checkpoint by transcriptionally upregulating the cyclin-dependent kinase (CDK) inhibitor p21. As a consequence, the kinase activity of several cyclin–CDK complexes is inhibited, preventing cell cycle transition from G1 to S phase[13]. After successful DNA repair, p53 releases the block on the G1/S transition and allows cell cycle progression.

As a last resort after irreversible cellular damage, p53 triggers signalling pathways that lead to the induction of apoptosis. Several pro-apoptotic proteins — including the B cell lymphoma 2 (BCL-2) family proteins PUMA (also known as BBC3), NOXA (also known as PMAIP1) and BAX — are transcriptionally activated by p53. At the same time, transcriptional repression of the anti-apoptotic gene survivin (also known as *BIRC5*) and subsequent caspase activation have also been reported[14]; caspases are a family of aspartate-specific cysteine proteases that, upon activation, initiate and orchestrate apoptotic cell death. Furthermore, p53 is involved in the activation of death receptor 5 (DR5; also known as TNFRSF10B (the death domain-containing receptor for TNF-related apoptosis-inducing ligand (TRAIL)) and the pro-apoptotic genes BH3-interacting domain death agonist (*BID*), p53-induced death domain protein (*PIDD*) and apoptotic peptidase-activating factor 1 (*APAF1*)[15], which all participate in the induction of apoptosis. Aside from its transcriptional activity, a cytoplasmic pool of p53 directly translocates to mitochondria, where p53 induces oligomerization of the pro-apoptotic proteins BAX and BCL-2 homologous antagonist/killer (BAK) in the outer mitochondrial membrane, thereby causing the release of caspase-activating pro-apoptotic factors. In addition, p53

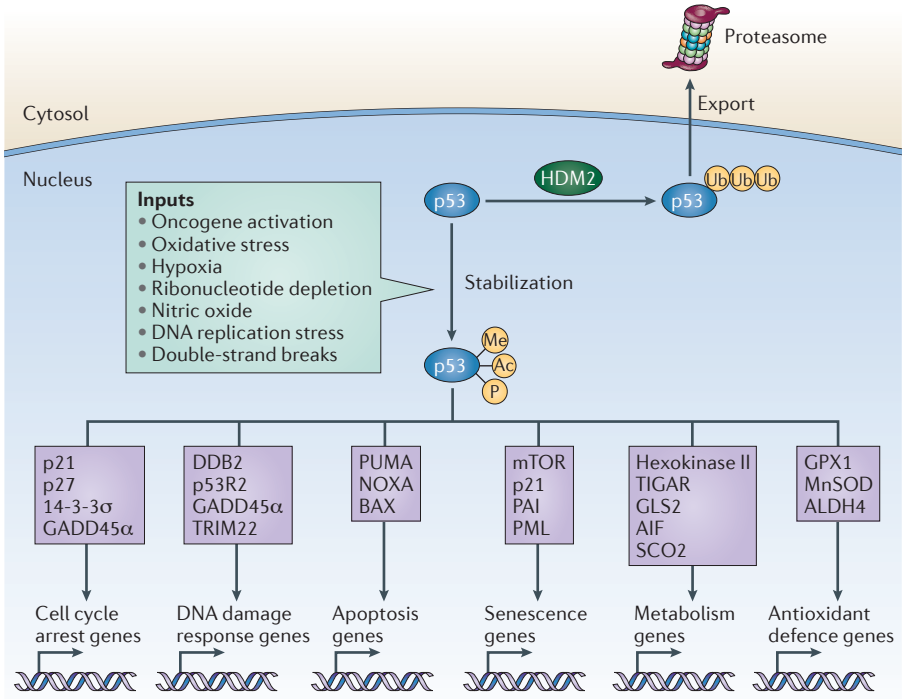

Figure 1 | **p53 pathways.** The tumour suppressor p53 has many functions and has roles in genomic stability control, apoptosis, metabolism and antioxidant defence. p53 is polyubiquitylated by the E3 ubiquitin ligase human double minute 2 (HDM2), which results in its proteasomal degradation. However, in response to certain extracellular and intracellular stimuli (such as oxidative stress, hypoxia, oncogene activation and DNA damage), p53 becomes post-translationally modified and stabilized. p53 can activate multiple pathways in response to cellular stress, through activation or repression of genes encoding a wide range of regulatory proteins. Depending on the severity of DNA damage, p53 can induce cell cycle arrest, senescence or apoptosis (which additionally involves direct protein–protein interactions of p53 with apoptotic proteins in the cytoplasm), and several metabolic pathways, including glycolysis, the pentose phosphate pathway and oxidative phosphorylation, are also regulated by p53. Moreover, p53 upregulates antioxidant defence genes encoding reactive oxygen species (ROS)-removing enzymes that are important for cellular and genetic stability and thus contribute to the antitumour function of p53. Ac, acetylation; AIF, apoptosis-inducing factor; ALDH4, aldehyde dehydrogenase 4; DDB2, DNA damage-binding protein 2; GADD45α, growth arrest and DNA damage-inducible protein 45α; GLS2, glutaminase 2; GPX1, glutathione peroxidase 1; Me, methylation; MnSOD, manganese superoxide dismutase; mTOR, mammalian target of rapamycin; P, phosphorylation; p53R2, p53-inducible ribonucleotide reductase small subunit 2-like protein (also known as RRM2B); PAI, plasminogen activator inhibitor; PML, promyelocytic leukaemia; SCO2, synthesis of cytochrome *c* oxidase 2; TIGAR, TP53-induced glycolysis and apoptosis regulator; TRIM22, tripartite motif-containing protein 22; Ub, ubiquitylation.

binds to and blocks the anti-apoptotic function of BCL-2 and BCL-X_L (also known as BCL-2L1)[16], which also results in the disruption of the outer mitochondrial membrane and the release of pro-apoptotic factors[17].

Intracellular pathogens, not unlike tumour cells, face the problem of obtaining sufficient energy and nutrients from their host for successful growth. *Shigella flexneri*, *Chlamydia trachomatis* and *Salmonella enterica* subsp. *enterica* serovar Typhimurium, for instance, are known to exploit the resources of their host, and it is increasingly being recognized that they modulate and redirect host metabolic pathways to do so[18–20]. Recently, it was discovered that p53 restricts the development and replication of intracellular bacteria by inhibiting the pentose phosphate pathway (PPP) in host cells[21], which demonstrated the importance of host cell metabolism for bacterial growth. As p53 controls several host metabolic pathways, interfering with p53 activity or activation seems to be a crucial event for all bacterial pathogens that replicate intracellularly.

In this Progress article, we discuss the mechanisms of p53 activation during infections caused by several facultative and obligate intracellular bacteria. To avoid induction of apoptosis in response to DNA damage, the production of reactive oxygen species (ROS) and other cellular stresses, bacteria such as *Helicobacter pylori*, *C. trachomatis*, *S. flexneri* and *Neisseria gonorrhoeae* mediate efficient degradation of p53 through the cellular calpain or ubiquitin–proteasome system. It is of particular interest that degradation of p53 is crucial for the bacterial infection process, delaying host cell death and providing sufficient metabolic support to facilitate bacterial growth.

Bacterial modulation of p53

It is becoming increasingly evident that bacterial pathogens, especially those with an intracellular life cycle, manipulate the human host cell to ensure their own survival. Cytoskeletal rearrangements, induction of anti-apoptotic pathways and control of the host cell cycle are all used to benefit bacterial infection and growth. However, bacterial pathogens also need to counteract the severe damage that infections often cause to the host if they are to avoid the immediate loss of their replication niche. For example, infection with *H. pylori* causes DSBs in host chromosomes, triggering DNA repair involving the ATM-dependent recruitment of 53BP1 as well as phosphorylation of H2AX[22]. Similarly, cells infected with *Listeria monocytogenes* show increased levels of DSBs. Although DNA damage responses are activated, the listerial toxin listeriolysin O dampens signalling pathways through degradation of the DNA damage sensor MRE11 (REFS 23,24). In the same way, infections with *S. flexneri* or *N. gonorrhoeae* were demonstrated to cause strong genotoxic stress[25,26]. Production of ROS during infections with the obligate intracellular pathogen *C. trachomatis* was found to contribute to DSBs, as shown by the phosphorylation of H2AX and methylation of histone H3. Similarly to *L. monocytogenes*, *C. trachomatis* interferes with DNA repair in the host cell by inhibiting the recruitment of phosphorylated ATM (pATM) and 53BP1 (REF. 27).

As DNA damage and ROS are strong inducers of p53-mediated apoptosis, bacterial pathogens have evolved multiple strategies to bypass p53 signalling. Notably, manipulation of p53 signalling has previously been recognized as an important mechanism used by viruses and protozoan parasites to ensure host cell survival (BOX 1), highlighting the importance of this pathway for intracellular pathogens.

Helicobacter pylori. *H. pylori*, a Gram-negative pathogen that colonizes the stomach of half of the world's population, has long been known to increase the risk of developing stomach cancer, especially gastric adenocarcinoma (BOX 2). At a genetic level, patients with gastric cancer who were

infected with *H. pylori* strains that harbour the cytotoxin-associated gene (*cag*) pathogenicity island (PAI), which encodes the virulence factor CagA, exhibited more severe inflammation of the gastric epithelium than those infected with CagA-negative *H. pylori* strains. Furthermore, tumours in patients infected with CagA-positive strains are significantly more likely to have mutations in *TP53* than tumours in patients infected with CagA-negative strains[28]. CagA is delivered into epithelial cells by a type IV secretion system after bacterial attachment and functions as a bacterial oncoprotein (FIG. 2). After delivery into the host cell, it activates several intracellular signalling cascades, leading to cellular morphological changes and an alteration of the apoptotic response. Activation of phosphoinositide 3-kinase (PI3K)–AKT signalling in gastric epithelial cells requires the *cag* secretion system and the cell wall component peptidoglycan in the *H. pylori* cell, as well as epidermal growth factor receptor (EGFR) transactivation and SRC activation in the host cell. Activated PI3K initiates phosphorylation of the serine/threonine kinase AKT1 and subsequent phosphorylation of HDM2, which in turn catalyses the formation of polyubiquitin chains on p53, marking it for proteasomal degradation[29]. CagA also directly associates with apoptosis-stimulating of p53 protein 2 (ASPP2) to initiate proteasomal degradation of p53 in an ASPP2-dependent manner[30]. Thus, CagA increases the survival of gastric epithelial cells that have sustained DNA damage during *H. pylori* infection[22]. However, *H. pylori* was also demonstrated to increase levels of p53 in the gastric mucosa of Mongolian gerbils 4–6 h after infection, although this was followed by a rapid decrease. A second peak of p53 upregulation — probably driven by infection-induced DNA damage — occurred several weeks later, which might be expected to enhance apoptosis in mucosal cells. This increase in the level of p53 was accompanied by inflammation, which is thought to increase the risk of gastric cancer in infected individuals[29,31].

In addition to increasing p53 degradation, *H. pylori* directly contributes to p53 mutagenesis by inducing the expression of activation-induced cytosine deaminase (also known as single-stranded DNA cytosine deaminase), a DNA- and RNA-editing enzyme that has been associated with *TP53* hypermutation and gastric cancer[32]. Finally, a third strategy by which *H. pylori* manipulates p53 has recently been shown, whereby the pathogen shifts the expression profile of p53 towards inhibitory isoforms that have truncated amino termini[33]. During *H. pylori* infections, activation of the transcription factor activator protein 1 (AP1), which is mediated by host–bacterium interactions through the *cag* PAI, and phosphorylation of JUN lead to the expression of N-terminally truncated p53. These isoforms were suggested to promote tumorigenesis, as they inhibit the transcriptional activity of wild-type p53, induce nuclear factor-κB (NF-κB) activity and increase survival of infected cells[33].

Chlamydia trachomatis. The obligate intracellular pathogen *C. trachomatis* relies on the host for nutritional support, including nucleotides, amino acids, lipids and sugars. An active role for *C. trachomatis* in controlling metabolite availability has recently been shown in a study that demonstrated the degradation of p53 during the early stages of infection as a means of reprogramming cellular metabolic pathways to generate a favourable environment for the pathogen[21]. Destabilization of p53 was initiated in a similar way as during *H. pylori* infections: after activation of the PI3K–AKT signalling pathway, HDM2 was phosphorylated and activated, resulting in proteasomal degradation of p53. The PI3K–AKT pathway has been linked to the inhibition of apoptosis during *C. trachomatis* infections, facilitating stabilization of the anti-apoptotic protein myeloid leukaemia cell differentiation protein 1 (MCL1) and sequestration of the pro-apoptotic protein BAD[34,35]. *C. trachomatis* engages the receptor tyrosine kinases (RTKs) ephrin receptor A2 (EPHA2)[36] and EGFR[32] to activate the PI3K–AKT pathway (FIG. 2); however, a role for RTK activation in the control of p53 stability in infected cells remains to be shown. Studies have shown that stabilization and activation of p53 by the anticancer drugs Nutlin-3 and etoposide resulted in strong inhibition of chlamydial growth owing to impaired inclusion formation[21] and a consequent inability of the pathogen to complete its life cycle, which led to a dramatic loss of infectivity.

Box 1 | **Manipulation of p53 by viruses and parasites**

The tumour suppressor p53 defends host cells against infection through the regulation of cell cycle progression and apoptosis. As with intracellular bacteria, replication of DNA viruses, which takes place in host cell nuclei, usually induces the cellular DNA damage response and activation of p53. To prevent the inhibition of viral replication, many viruses manipulate p53 by promoting its degradation or suppressing its transcriptional activity. This also allows cell cycle transition of quiescent cells into S phase, ensuring efficient viral replication.

For example, the E6 oncoprotein encoded by human papillomavirus type 16 (HPV-16) and HPV-18 promotes the degradation of p53 by the ubiquitin–proteasome system[56]. Other viruses, including adenovirus, Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), also use the ubiquitin–proteasome system to inhibit p53. Adenoviral proteins E4orf6 and E1B 55K cooperatively target p53 to induce its ubiquitylation and efficient proteasomal degradation[57]. The EBV protein BZLF1 acts as an adaptor of the ECS (Elongin B/C–CUL2/CUL5–SOCS-box protein) ubiquitin ligase complex, which also targets p53 for proteasomal degradation[58]. The viral interferon regulatory factor 1 (vIRF1) of KSHV interacts with p53 and causes its downregulation by ubiquitylation, which facilitates proteasome-mediated degradation[59]. Furthermore, the latency-associated nuclear antigen (LANA) of KSHV interacts with p53 and inhibits its transcriptional activity, thus contributing to viral persistence and oncogenesis[60]. This strategy of inhibiting the transcriptional activity of p53, rather than initiating its degradation, is also used by other viruses, including hepatitis B virus (HBV), in which the viral protein HBV-encoded X antigen (HBxAg) blocks the transcriptional activity of p53 by binding to its amino-terminal region[61]. Simian virus 40 (SV40) and the RNA viruses human T lymphotropic virus (HTLV), hepatitis C virus (HCV) and HIV were also shown to downregulate p53: HTLV inactivates p53 by blocking its transactivation function, the large T antigen of SV40 and NS5A of HCV physically interact with p53 and inhibit p53-mediated transcription, and the HIV protein Tat inhibits the transcription of p53 (REFS 62–65).

The mechanism of targeting p53 for proteasomal degradation used by the bacterial pathogens *Helicobacter pylori*, *Chlamydia trachomatis* and *Shigella flexneri*, in which the activation of phosphoinositide 3-kinase (PI3K)–AKT signalling induces p53 ubiquitylation by human double minute 2 (HDM2)[66], is shared with the RNA virus respiratory syncytial virus (RSV). Interestingly, HDM2 is also activated by the malaria parasite *Plasmodium yoelii*, which substantially decreases p53 protein levels during its liver-stage infection. Pharmacological stabilization of p53 by the anticancer drug Nutlin-3 led to a strongly reduced liver-stage burden of the parasite, emphasizing the importance of p53 modulation for successful liver-stage infection[53]. The closely related cattle parasite *Theileria annulata*, which is transmitted by ticks, induces cytoplasmic sequestration of p53, thus blocking p53-mediated apoptosis[67].

Box 2 | ***Helicobacter pylori* and gastric cancer**

Almost 16% of cancer cases worldwide are caused by infectious diseases, mostly due to viruses. However, bacterial infections may also increase the risk of developing cancer: for example, *Helicobacter pylori* infections are strongly associated with gastric carcinoma.

Gastric cancer is the second leading cause of cancer-associated death worldwide. Two types of gastric cancer can be distinguished: gastric cardia cancer, which affects the top inch of the stomach, and non-cardia gastric cancer, which occurs in all other regions of the stomach. Although specific dietary factors, smoking and genetic predispositions increase the risk of gastric cancer, *H. pylori* infections are the most common cause, accounting for 60–75% of cases. Epidemiological studies have shown that the risk of developing non-cardia gastric cancer is almost sixfold greater in patients who are infected than in non-infected individuals[68]. Cohort studies comparing patients with non-cardia gastric cancer and cancer-free participants further supported these findings, concluding that *H. pylori*-infected individuals had a nearly eightfold increased risk of developing non-cardia gastric cancer[69]. Chronic and acute inflammation of the gastric mucosa after activation of innate and acquired immune responses during *H. pylori* infections, and secretion of pro-inflammatory cytokines and interleukins (ILs), especially tumour necrosis factor (TNF) and IL-6, are thought to strongly contribute to chronic gastritis and cancer. In rare cases, colonization with *H. pylori* results in the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma; the risk is sixfold greater in infected than in non-infected individuals[70].

Expression of the bacterial effector gene cytotoxin-associated gene A (*cagA*) and its injection into gastric epithelial cells by a type IV secretion system was found to be specifically associated with the development of non-cardia gastric cancer. CagA efficiently disrupts the apical junctions of epithelial cells and impairs epithelial cell differentiation, creating a pro-invasive environment for *H. pylori*[71]. Individuals carrying CagA-positive *H. pylori* strains are twice as likely to develop non-cardia gastric cancer than individuals infected with CagA-negative *H. pylori* strains[72]. Injection of CagA into host cells activates several signalling pathways, including proliferation, survival and anti-apoptotic pathways mediated by phosphoinositide 3-kinase (PI3K)–AKT signalling. PI3K–AKT signalling and continued activation of AKT is found in advanced stages of gastric cancer[73]. In 2010, a study demonstrated the inactivation and manipulation of the tumour suppressor p53 by CagA, highlighting the connection between the bacterial effector and carcinogenesis[27]. Also important in gastric cancer development is the epithelial cadherin (E-cadherin) gene (*CDH1*), which is frequently mutated in hereditary diffuse gastric carcinoma (HDGC)[74]. A recent study demonstrated that co-culture of gastric epithelial cells with *H. pylori* leads to downregulation of E-cadherin. Although E-cadherin reduction might be expected to induce cellular stress and activation of p53, this is prevented by the human double minute 2 (HDM2)-mediated degradation of p53 that is activated by *H. pylori*[75].

In addition to PI3K–AKT signalling, *C. trachomatis* manipulates the regulation of the host PPP by p53. *C. trachomatis* was demonstrated to consume large amounts of the cellular NADPH pool, which is primarily generated by the PPP[37], and is auxotrophic for nucleotide precursors that are also synthesized by the PPP[38], which shows the importance of this metabolic pathway for the pathogen. p53 not only inhibits glycolysis but also the PPP[39], and thus strongly suppresses these pathways after overexpression. Interestingly, overexpression of glucose-6-phosphate dehydrogenase (G6PD), the first enzyme of the PPP, enabled recovery of chlamydial growth in the presence of high amounts of active p53, which suggests that downregulation of the PPP is a major mediator of the antibacterial activity of p53. This revealed a new role for p53 manipulation by *C. trachomatis*, in which the primary function of p53 destabilization was to facilitate metabolite supply, and thereby bacterial replication, rather than to ensure survival of the host cell. Furthermore, the dependence of the pathogen's growth on a functional HDM2–p53 axis emphasizes a potentially carcinogenic role for *C. trachomatis* infections[40]. It has even been suggested that p53 degradation during *C. trachomatis* infection has a direct role in the development of ovarian cancer, as functional p53 signalling is considered to be essential for preventing tumorigenesis in response to the cytotoxic stress triggered in fallopian tubes during ovulation[40,41].

Shigella flexneri. *S. flexneri* is a Gram-negative enteropathogen that in humans causes bacillary dysentery, which is characterized by fulminate inflammation and destruction of intestinal tissue. The pathogen is particularly harmful to its host: during the early stages of infection, *S. flexneri* induces strong genotoxic stress (that is, DSBs)[25], which leads to ATM phosphorylation and formation of γH2AX, both of which are indicative of the activation of the DNA damage response. Furthermore, *S. flexneri* infection results in necrotic cell death, owing to the loss of mitochondrial inner membrane potential that results from the activation of BCL-2–adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), a pro-apoptotic protein, and cyclophilin D (also known as PPIase F), a regulator of mitochondrial integrity[42]. As a countermeasure, *S. flexneri* prevents caspase 3 activation and activates a NOD1-dependent RIP2–IKKβ–NF-κB signalling pathway during the early stages of infection, which delays apoptotic and necrotic cell death in its host[42,43].

As with *H. pylori* and *C. trachomatis*, *S. flexneri* also destabilizes p53 by manipulating PI3K–AKT signalling (FIG. 2). During the early stages of infection, the *S. flexneri* virulence factor IpgD promotes a strong reduction in the amount of p53 through the activation of HDM2. IpgD is a phosphatase that generates phosphatidylinositol-5-monophosphate, which is a known activator of PI3K–AKT signalling and thus HDM2 phosphorylation. However, a second mechanism of p53 degradation is used during the later phase of infection: calpain, a calcium-activated neutral protease, cleaves p53 at a site within the N terminus of the protein, which results in an extremely unstable truncated form of p53 that is rapidly degraded by the proteasome[25] (FIG. 2). The calcium-dependent activation of calpains during *S. flexneri* infections has been demonstrated by measuring the autolytic maturation process, in which calpain is activated by cleavage of its regulatory subunit, CAPN4 (also known as CSS1). *S. flexneri* infection caused a marked reduction in the level of calpastatin, the endogenous inhibitor of calpains, which induced calpain activation[25]. It is thought that the type III secreted virulence effector VirA mediates this reduction by promoting calpastatin proteolysis. VirA shares homology with the enteropathogenic *Escherichia coli* virulence effector EspG, which was reported to activate calpain through an unknown mechanism[44], and an inducible expression experiment showed that VirA triggers calpain activity[25]. In parallel, it was shown that VirA is responsible for the calcium-dependent proteolysis of calpastatin[25], although whether this enzymatic reaction is mediated directly or indirectly by VirA is not known. Calpain activation led to the formation of p53 cleavage products, which could be prevented by depletion of *CAPN4* (REF. 25). The authors then demonstrated that prevention of p53 destabilization leads to an NF-κB-dependent induction of apoptosis in cells infected with *S. flexneri*. Moreover, calpain also

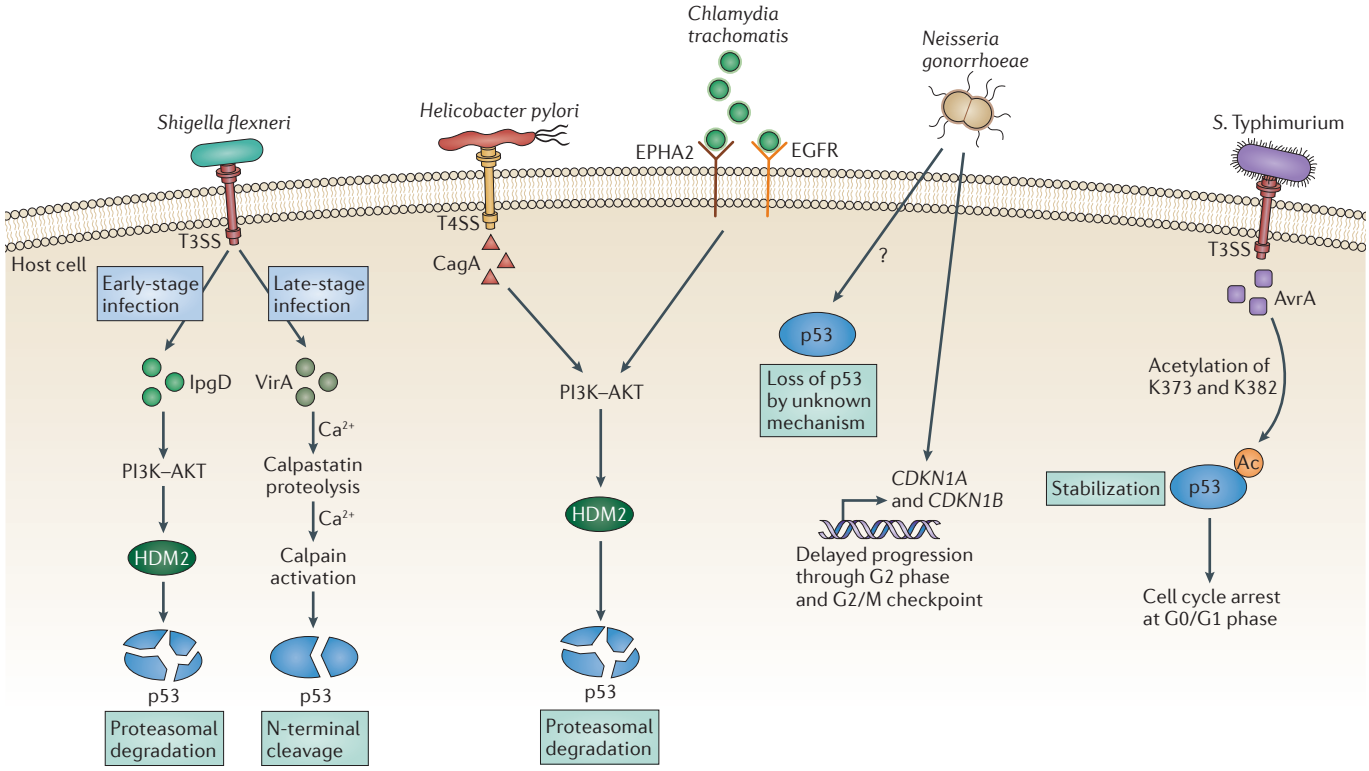

Figure 2 | **Bacterial modulation of p53.** To prevent the induction of apoptosis and other p53-regulated pathways that are detrimental to bacterial growth and dissemination, bacterial pathogens such as *Shigella flexneri*, *Helicobacter pylori*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* use different strategies to deactivate p53. *S. flexneri* uses both the cellular calpain system and phosphoinositide 3-kinase (PI3K)–AKT signalling to induce degradation of p53. In the early stages of infection, the *S. flexneri* effector IpgD induces proteasomal degradation of p53 through human double minute 2 (HDM2), which is activated by PI3K–AKT signalling. In the later stages of infection, another effector, VirA, induces proteolysis of calpastatin, a host protein that inhibits calpain. As a result of this loss of inhibition, calpain degrades p53 by cleaving its amino terminus. The *H. pylori* effector cytotoxin-associated protein A (CagA) induces PI3K–AKT signalling to activate HDM2, which results in proteasomal degradation of p53, whereas *C. trachomatis* also activates the PI3K–AKT pathway, but does so by engaging ephrin receptor A2 (EPHA2) and epidermal growth factor receptor (EGFR) at the host cell surface. *N. gonorrhoeae* downregulates protein levels of p53, but the mechanism by which it does so is still poorly understood, and also delays progression through the cell cycle by upregulating p21 and p27 (encoded by *CDKN1A* and *CDKN1B*, respectively), most likely at the transcriptional level. In contrast to these pathogens, the *Salmonella enterica* subsp. *enterica* serovar Typhimurium effector AvrA induces acetylation of p53, which is a stabilizing modification that is associated with cell cycle arrest in infected cells. Ac, acetylation; T3SS, type III secretion system; T4SS, type IV secretion system.

has a p53-independent role in promoting *S. flexneri* infection, as calpain inhibition prevented the actin reorganization required for bacterial entry and thus reduced the number and size of *S. flexneri* entry foci[25].

In contrast to these findings, in which *CAPN4* depletion perturbed *S. flexneri* infection, a massive increase in bacterial proliferation in *CAPN4*-deficient host cells has also been reported[25]. Furthermore, *CAPN4* inactivation during infection markedly delayed the onset of morphological changes that are indicative of cell necrosis. Thus, the activation of calpain in this context is a double-edged sword: it supports entry of *S. flexneri* into the host cell and inactivates p53 to prevent early apoptosis, but it also shifts cell fate to a necrotic type of death[25] that controls bacterial replication over a longer timescale[45]. However, of the two cell fates, necrosis is preferable to *S. flexneri*, as p53-induced apoptosis would lead to a more rapid destruction of its epithelial niche and to the removal of apoptotic cells with their bacterial content by phagocytes.

Other examples. *N. gonorrhoeae* is a Gram-negative human pathogen that is the cause of the sexually transmitted disease gonorrhoea, which affects more than 100 million individuals worldwide each year. As *N. gonorrhoeae* infections are mostly asymptomatic, infected individuals often carry the disease for months. In severe cases, *N. gonorrhoeae* infections can lead to pelvic inflammatory disease, ectopic pregnancy or infertility. *N. gonorrhoeae* infection has been demonstrated to result in severe damage to host cell DNA, including DSBs and single-strand breaks, as indicated by the presence of 53BP1-positive foci and γH2AX in infected human non-tumour vaginal VK2/E6E7 and human cervical carcinoma ME-180 cell lines[26]. Despite this severe genotoxic stress, both mRNA and protein levels of p53 were decreased in VK2/E6E7 cells during gonococcal infections, although the mechanism underlying this decrease is still unknown and requires further clarification. However, p53 levels were neither increased nor reduced in ME-180 cells, indicating that the infection-induced genotoxic response can differ between cell types. p53-independent host cell responses to genotoxicity are also observed in cells infected with *N. gonorrhoeae* and represent a further challenge to replication by the pathogen. Specifically, expression of the CDK inhibitors p21 and p27 was increased after infection, which is in line with a decelerated cell cycle and reduced cell proliferation[26], and progression through the G2 phase and G2/M checkpoint was delayed (FIG. 2).

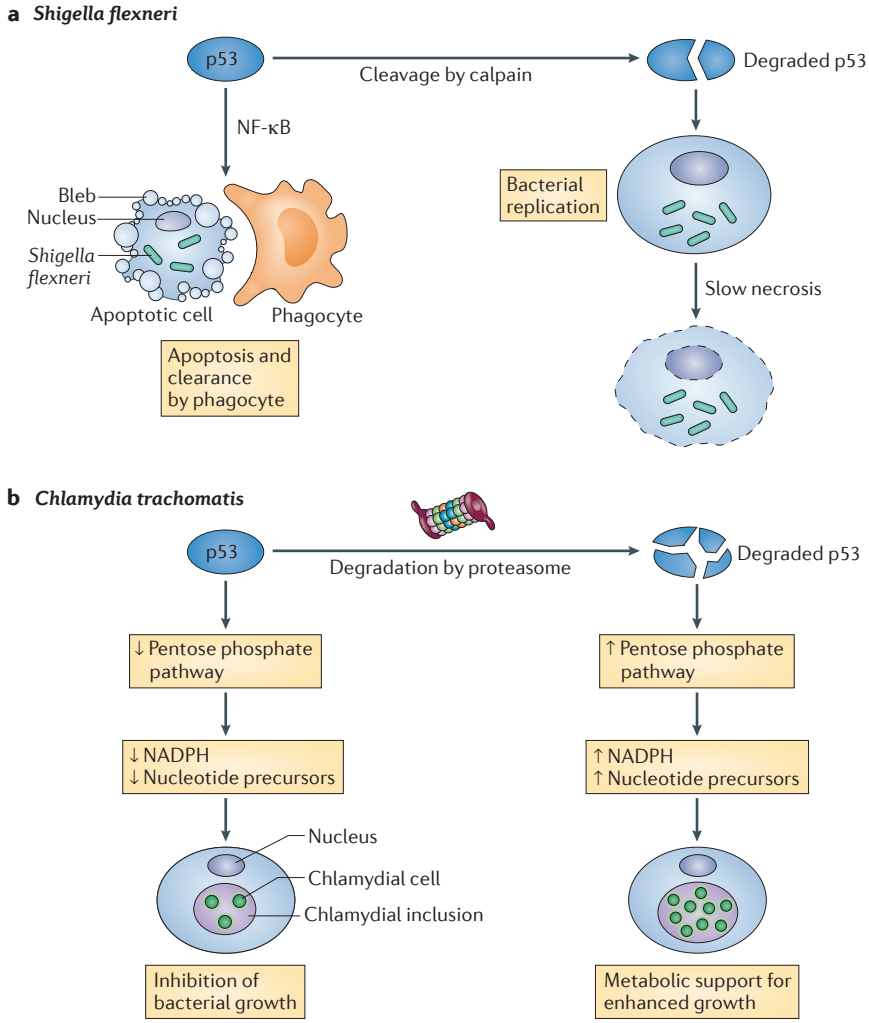

Figure 3 | **Consequences of p53 modulation for the infection process.** Pathogen-induced degradation of p53 is expected to have profound repercussions for the bacterial infection process. **a** | *Shigella flexneri* induces the degradation of p53 through calpain proteases at the cost of the induction of necrosis, which reduces bacterial proliferation. As necrosis is a slower process than p53-mediated apoptosis, the degradation of p53 nevertheless prolongs survival of the pathogen's niche. **b** | *Chlamydia trachomatis* infection represents an interesting example of bacterial manipulation of cellular metabolism. Degradation of p53 during *C. trachomatis* infection activates the pentose phosphate pathway and, as a result, enhances the rate of biosynthesis of the reducing agent NADPH, high levels of which are consumed by the pathogen during growth, and of nucleotide precursors for which *C. trachomatis* is auxotrophic. Conversely, increased levels of p53 deplete the levels of these metabolites, and thus inhibit bacterial growth and lead to the loss of chlamydial infectivity. NF-κB, nuclear factor-κB.

In contrast to the pathogens described above, which suppress p53 signalling, the Gram-negative facultatively anaerobic bacterium *S.* Typhimurium activates p53 signalling pathways (FIG. 2). The *S.* Typhimurium type III secreted virulence factor AvrA, which is a multifunctional deubiquitinase with acetyltransferase activity towards specific mitogen-activated protein kinase kinases, has been shown to be responsible for this activation. In a cell-free transacetylase assay, AvrA acetylated purified p53 at lysine 373 and lysine 382 (REF. 46). Acetylation of these residues promotes an open conformation of p53 and thereby enhances its transcriptional activity[47]. As expected, expression of bacterial AvrA strongly increased the transcriptional activity of p53 in the human intestinal epithelial cell line HCT116, leading to cell cycle arrest at G0/G1 phase[46], and co-transfection of p53 and AvrA constructs demonstrated a physical interaction between the two proteins. AvrA also inhibits JUN N-terminal kinase (JNK) and NF-κB signalling pathways, but the effect of this inhibition on p53 signalling has not been investigated. In addition to HCT116 cells, p53 acetylation following *S.* Typhimurium infection has been demonstrated in human colonic epithelial cells (HT29 cl.19A and Caco-2 BBE), in mouse embryonic fibroblasts and in mouse colonic epithelial cells[46].

Currently, it is unclear how p53 activation supports *S.* Typhimurium infection. However, infection-induced cell cycle arrest and inflammatory responses are hallmarks of *S.* Typhimurium infection, and these infection outcomes are, at least in part, dependent on p53 activation.

Consequences for the infection process

Despite our extensive knowledge of the multiple functions of p53, whether p53 is involved in the host defence against bacterial infections remains unknown. Currently, three possible strategies by which p53 interferes with bacterial infection are postulated: modulation of the immune response, cell death and cell metabolism control. p53 has been shown to be a suppressor of pro-inflammatory pathways, repressing the expression of interleukins (ILs) and other inflammatory cytokines and NF-κB signalling[48–50]. Furthermore, p53 has been reported to regulate genes that encode pathogen recognition factors, such as Toll-like receptor 3 (TLR3)[51], chemokines and immune cell activation factors, including colony-stimulating factor 1 (CSF1), monocyte chemotactic protein 1 (MCP1; also known as CCL2), chemokine C-X-C motif ligand 1 (CXCL1; also known as GROα) and IL-15 (REF. 52). Most of these studies were carried out *in vitro* in immune cells, such as neutrophils or macrophages, by stimulating either p53-deficient cells or cells in which p53 had been chemically stabilized using activating substances (such as lipopolysaccharide, IL-2 and IL-4). However, a role for p53 in the antibacterial immune response has not yet been demonstrated in an animal model.

Currently, there is more evidence for the antibacterial functions of p53 in non-immune cells than in immune cells, and these have been documented in several cases. As explained above, the damage to the host DNA elicited by bacterial infections, which is sometimes irreparable, induces host signalling pathways that the pathogen modulates, in particular to suppress p53 function. This ensures the inhibition of p53-induced cell death or at least extends the lifespan of the host. *S. flexneri* infections are an excellent example of bacterial strategies that prolong host survival by interfering with p53 levels. As explained in detail above, the *S. flexneri*

effector VirA activates the cleavage of p53 to prevent the induction of apoptosis and thus ensure the prolonged survival of the pathogen's epithelial niche (FIG. 3a).

In addition, downregulation of p53 may redirect cellular metabolism in a way that is beneficial for bacterial growth. Shaping the cellular environment for optimal bacterial replication is of crucial importance for successful dissemination of the pathogen and is therefore under high evolutionary pressure. This has been demonstrated for *C. trachomatis*, which relies on nutritional support from its host and, as explained above, uses the effect of p53 degradation on metabolic pathways to support bacterial growth as efficiently as possible (FIG. 3b).

The examples described in this Progress article from several bacterial pathogens, in which the pathogen induces degradation of p53 or modulates it in other ways during infection, support the hypothesis that p53 has an antibacterial function. As outlined above, pharmacological stabilization of p53 led to severe growth defects in and a complete loss of infectivity of *C. trachomatis*. The same effects have been observed in the parasite *Plasmodium yoelii*, in which increased p53 levels resulted in a reduced infection rate and liver-stage burden[53] (BOX 1). Considering the fundamental role of p53 in the regulation of metabolism, it is likely that most pathogens with an intracellular phase of replication require inactive or downregulated p53, and thus manipulation of p53 signalling is expected to be relevant for a broad range of pathogens, and not only those that induce oxidative stress or cause DNA damage. Recent evidence suggests that multiple forms of metabolic stress can induce p53 activation, including glucose deprivation, a low NAD/NADH ratio and a low AMP/ATP ratio. In response to sensing metabolic stress, p53 downregulates PI3K–AKT and mammalian target of rapamycin (mTOR) signalling pathways, which reverses the high metabolite consumption status of the cell and induces autophagy, a cellular rescue programme[13]. This metabolic shift would limit nutrient availability for pathogens growing inside cells, suggesting that metabolic regulation by p53 provides a host defence against infection that must be overcome by the pathogen. Indeed, mechanisms to overcome p53 activation or activity may be required for any form of infection-induced changes in the cellular energy state or level of metabolite consumption, which almost certainly will occur as a consequence of the intracellular replication of bacterial pathogens.

Conclusions

The observations and ideas discussed in this Progress article suggest a role for p53 in controlling bacterial infection both by limiting survival of the infected cells and by directly restricting intracellular replication of the bacteria. Interfering with p53 is thus an essential step for these pathogens to extend the lifespan of the host cell and to access sufficient nutrients during intracellular replication. Targeting of a tumour suppressor such as p53, however, compromises the natural safety system of a cell, as unrestricted cellular growth and an impaired DNA repair programme promote mutations and tumorigenesis. Even so, despite the fact that bacterial infections are widespread, a bacterial origin of carcinogenesis is still not generally acknowledged, although the association of *H. pylori* infection with gastric cancer and several other recent examples have tremendously boosted the recognition of this topic[54,55]. In the latter cases, the DNA damage-inducing agents produced by bacteria have been demonstrated to be the cause of cancer development. If one considers bacterial infection as the source of both DNA damage and the downregulation of p53, the promotion of tumorigenesis by bacterial infection may be of much broader significance than anticipated thus far. Conversely, as in the case of *S.* Typhimurium, p53 may be activated during infection and favour infection-associated pathologies such as inflammation.

Therapeutic targeting of p53 may offer an interesting new approach in the treatment of intracellular pathogens, as an alternative to existing therapies that, for at least some bacteria, are losing potency owing to the rapid spread of antibiotic resistance. However, more research is required to elucidate this fascinating interplay of microbial infection and tumour control.

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Competing interests statement
The authors declare no competing interests.