The cells safety equipment is called p53

Sonja Buratovic
Populärvetenskaplig sammanfattning av Självständigt arbete i biologi 2009
Institutionen för biologisk grundutbildning, Uppsala Universitet

Imagine yourself going for a ride on a rollercoaster that didn´t have any breaks or safety equipment. You would not do that would you? To be able to enjoy a thrilling ride you would have to be sure that it was safe and that no harm could come your way. Just as breaks and seatbelts provide this security on a rollercoaster p53 ensures the safety and integrity of our cells. With a complex mechanism of sensing the environment around the cell and ensure that no damage could slip through the many passages of the cell cycle the p53 can be viewed as a protector of the genome. If the protector senses damage in the genome it will immediately step on the brakes and help other proteins make the corrections needed for the cell to be able to precede the ride through the cell cycle unharmed. But as we all know no rollercoaster is a 100 percent safe and actually p53 also isn´t able to correct all damages that a cell can be put up to. When it fails to do so we get struck with the feared disease called cancer.

Investigating the p53 protein from 1979 until today

p53 protein was first described in the literature as early as 1979 but at that time scientists didn´t really know what function it had in the cell. The first studies performed concluded that rodent cells which were infected with the DNA virus SV40 not only developed tumors but also expressed an earlier unknown 54 kDa big protein. The size of the activated gene product later gave name to both the gene and its protein product, p53. It was found that p53 was expressed in a great variety of cells derived from different donors such as humans, monkeys and hamsters. Now it was a fact that this protein must play a crucial role in the cell due to the fact that it was conserved in so many different species.

To figure out what function *p53* had in the cell it was expressed in untransformed cell lines and its product was closely monitored. These cells showed elevated levels of p53 and extensive stabilization of the protein when they were close to the G1 to S checkpoint in the cell cycle. Not surprisingly the levels of p53 were lowered if the cell cycle was inhibited. Other studies performed at the same time showed almost the same results and more importantly that p53 could inhibit the cells passage from G0 to S phase.
The thought of *p53* being a potential oncogene came when it was concluded that it could transform benign cells into a malign phenotype in a much similar way that known oncogenes like *myc* did. Problems with this assumption arose when transfection assays, using other *p53* sequences than the initially cloned cDNA, didn´t give rise to the foci one had expected. As a matter of fact this newly made *p53* sequence inhibited the action of the known potent oncogene *ras* when they were co-expressed. This finding lead to extensive sequencing of multiple different p53 and one eventually concluded that the gene was a tumor suppressor.

Cancer Glossary

Oncogene: We all have sets of different genes that control the cells growth and proliferation. Under normal circumstances these genes are called proto-oncogenes and are responsible for the quick turnover of epithelial cells like the ones in the gastrointestinal tract or skin. When a proto-oncogene is subjected to mutations it will transform and become an active oncogene. One well studied oncogene is the *H-ras* and when it is mutated by a single amino acid substitution in its 12^{th} codon, converting glycine to valine, it is known to give rise to bladder carcinomas. Another important oncogene is *Myc*. This gene can be subjected to many different kinds of mutations like amplification, pro-virus integration or translocation. It is easy to understand how gene amplification can cause elevated levels of the *Myc* gene product. In order for the other two kinds of mutations to have the same effect on the cell they need to somehow be linked to the genes promoter. Pro-viruses have their own promoters and thus are able to transform the proto-oncogene into a potent oncogene. Translocation of coding sequences of *Myc* to another gene with higher transcriptional activity e.g. an immunoglobulin promoter will elevate the rate of transcription.

Tumor suppressor gene (TSG): These very diverse genes have one common feature – the ability to halt cell growth and proliferation. Just like oncogenes tumor suppressor genes need to be mutated in order for tumors to form. In most cases both alleles of a TSG need to be mutated for it to lose its function. In most cases a mutation will strike just one allele but as a result of Loss of Heterozygosity (LOH), by mitotic recombination, also the other allele copy will be affected. Mutations in TSGs can be inherited and therefore only a single somatic mutation is needed to inactivate the gene in children's retinoblastoma.

p53 controls many different aspects of not only the cells life but also other gene outputs. It can bind to specific genetic sequences and depending on which gene it binds to it can either repress or activate the transcription. To be able to do that p53protein has to have different sites with different functions. Today one usually divides p53 into 4 domains, each having a specific function. If we start from the beginning of the protein transcript we find an important binding site for p53s own inhibitor MDM2. The levels of these two proteins are regulated by one another. If p53 levels rise it will bind to *MDM2* and thus activating transcription of the latter gene product. MDM2 can then bind to p53s first domain, the amino domain, and inhibit its function as a pro-apoptotic agent. As p53 levels decline so does the transcription of *MDM2* and an auto regulatory feedback loop is created. In conclusion, one protein would not function properly without the other.

These events are only true if the cell isn´t affected by any stress signals such as ionizing radiation, chemotherapeutic drugs or other DNA damaging agents. If that happens, p53 will dimerize with other p53 molecules using another of its four functional domains. This time it's the last end of the protein, the carboxy-terminal, that is used to keep the parts together. The next event in the "break machinery" is the binding of p53 to the damaged DNA. And for this purpose the central-, or as it is also called core-domain play an essential role. So now p53 has dimerized, bound to the DNA that need to be fixed and what next? Actually the following events depend on what kind of response p53 needs to trigger in the cell. If the damage is of such a kind that the cell only requires some extra time in the G1 phase to be able to repair itself p53 will bind to a sequence on chromosome 6 where the *WAF1* gene is located. This gene product will bind to kinases that normally give the "ok to go" signal for passage to S phase of the cell cycle and stop the progression, giving DNA repair molecules the much needed extra time to perform their work. p53 acts in a very similar way on another cell cycle inhibitor called *GADD45*.

As we all know not all DNA damage is repairable. But no need to worry! p53 controls the cells suicide program also. To use a more scientific expression I will not call it suicide but rather apoptosis program. This is controlled by not one but two proteins, which are actually related to one another, even though they have the total opposite effects on the cells life. The *Bcl-2* will promote survival and growth whereas *Bax* makes the cell more prone to undergo the apoptotic program. Elevated p53 levels will lead to the repression of *Bcl-2* and stimulation of *Bax* by binding to similar sequences in the genes promotors. Elevated levels of Bax will not only bind to Bcl-2 and repress its survival effects but also trigger the release of apoptotic molecules such as caspases from the mitochondria.

So if p53 is such a great protector of the cell and the genome why do we develop tumors? The answer to that question is that p53 is inactivated in most, not to say nearly all, human cancers. As a reflection of the complex nature of tumors the incidences that inactivate p53 are numerous. Most common are missense mutations in the *p53* and 91% of the mutations will occur in the core-domain coding sequence. These single amino acid substitutions will have a fatal effect on p53s ability to bind to the DNA. Another quite common mutation site is the carboxy-terminal which will then prevent p53 from binding to one another and thus it will not be able to bind to DNA. As mentioned earlier MDM2 has a negative effect on the p53s apoptotic abilities. When multiple copies of *MDM2* are present in the genome higher levels of its protein product will be translated and the consequence is that more p53 than normal is bound and inactivated.

The question that then arises is whether there can be something done to reactivate this inactive p53 and of course the answer is yes. Novel strategies for targeting p53 in gene therapy have evolved. The creation of a virus capable of infecting tumor cells and introducing a wild type p53 for transcription is now passing through the last stage of clinical trials and may soon be available as a therapy, probably combined with conventional therapies like radiation or chemotherapy. Much more research needs to be done in the field of gene therapy that targets p53 but this method might provide therapies that have far less negative side effects compared to the ones available today.

More information

Buratovic S. 2009. Genomets väktare – p53. Kandidatuppsats 15 hp, Uppsala universitet.

Vogelstein B, Lane D, Levine AJ. 2000. Surfing the p53 network. Nature **408**: 307-310