The search for new treatment strategies for malignant pleural mesothelioma

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English summary.

What is this thesis about?

This thesis is about finding new treatment options for patients with malignant pleural mesothelioma (MPM). MPM is a tumor of the mesothelial cells lining the pleural cavity. It is also known as asbestos cancer due to its association with asbestos. Even though all handling of asbestos is strictly regulated since 2005, still 500-600 patients per year are diagnosed with MPM in the Netherlands. This is caused by a latency period of 20-50 years between asbestos exposure and development of the tumor. These numbers are not expected to decrease in the coming years. The prognosis for these patients is poor and without treatment most patients die within a year. This stretched the need for new treatment options for MPM.

What is done?

Chapter 1 gives an overview of which new treatments have been tested over the last couple of years. First-line chemotherapy consists of a plantinum-based drug combined with pemetrexed. This combination gives a survival benefit of 16.1 months compared to patients that did not receive treatment. Around 40% of the patients respond to this combination. For patients that do not respond to first-line chemotherapy or become progressive after treatment, there is no standard second-line regimen. Many new treatments, like growth factor inhibitors, angiogenesis inhibitor, other targeted agents, oncolytic viral therapy and vaccines have been tested as second-line treatment. However, none of these therapies showed a significant survival benefit. That all these treatments fail in phase II clinical studies while active in preclinical studies shows the urgent need for better preclinical models that resemble the patients tumor, but are also easy to handle and fast in its readout. ***Chapter 1*** also describes the latest developments in preclinical models, like cell lines, primary tumor cultures and mouse models, and their own advantages and disadvantages.

Personalized treatment

In ***chapter 2*** I present a personalized treatment strategy based on primary tumor cultures. A method for screening multiple chemotherapies on the patients' own tumor cells is developed, to generate chemical profiles and select the best therapeutic option. For ten patients treatment decision was based on these chemical profiles. There was a strong correlation between the *in vitro* results and the actual tumor response in these patients, which indicates this personalized treatment strategy is possible in patients with MPM. Further validation is currently ongoing in a phase II trial named the PROOF study.

Based on the chemical profiles of all tumor cultures, these tumor cultures could be divided in three groups. Tumor cultures that respond to almost all tested chemotherapies so called 'responders', tumor cultures that did not respond to chemotherapy, so called 'non-responders' and a group of tumor cultures that was sensitive to some of the chemotherapies, but not too all, the so called 'intermediate responders'. When comparing these groups genetically, a gene expression profile that distinguished the 'responders' from the 'non-responders' was identified. The 'intermediate responders', showed a different unique genetic profile, which did at some levels overlapped with the 'responders' or 'non-responders' group. With these gene expression profiles we were able to identify the fibroblast growth factor receptor (FGFR) as a new target for the treatment of MPM.

FGFR

FGFR was not only found in the gene expression profiles of our primary tumor cultures. The screening of multiple new therapies on different MPM cells lines and primary tumor cultures, as described in ***chapter 3***, also showed that a subset of the cultures were sensitive to FGFR inhibitors. A MPM mouse xenograft model confirmed the sensitivity to FGFR inhibitors. The cultures sensitive to FGFR inhibitors showed elevated levels of FGF9 mRNA. FGF9 is known to have a high affinity for FGFR3. All the sensitive cell lines were dependent on FGFR3 mediated signaling which was regulated by BRCA-associated protein 1 (BAP1). Therefore BAP1 protein loss, could serve as a biomarker to select patients for FGFR inhibitor treatment.

Antibody drug conjugates

Another treatment strategy that is described in this thesis is antibody drug conjugates (ADCs). ADCs consist if a monoclonal antibody chemically conjugated to a potent cytotoxic drug. When the antibody binds the target antigen, the ADC will internalize into the cell and release the drug that will kill the tumor cell. When the target antigen is only expressed on tumor cells the drug will only kill the tumor cell and not affect the normal cells, giving fewer side effects. Expression on tumor cells and not on normal cells, internalization into the cell and releasing of the drug are all important factors in this therapy strategy.

Chapter 4 shows that throphoblast glycoprotein, or 5T4, is a suitable target for ADC treatment in MPM. The antigen is expressed in most of the MPM tumors and not expressed in normal tissue. Upon binding the whole complex internalizes into the cell. Two of the three ADCs that were tested were able to kill the tumor cells that had a strong expression of 5T4. One ADC was not able to kill the tumor cells. We showed that the released drug was trapped in the lysosomal compartment of the cells. By changing the pH of the cells with cloroquine,

an anti-malaria drug, this ADC was also able to kill the tumor cells.

BAP1

BAP1 is a molecular target that has been identified as a potential novel target in the treatment of MPM. BAP1 is a tumor suppressor gene regulating target genes in transcription, cell cycle control, DNA damage repair and cellular differentiation. Somatic mutations in BAP1 are seen in 47-67% of the patients with MPM. ***Chapter 5*** describes how therapeutic agents could reverse phenotypic effects of BAP1 protein loss. However since the exact molecular mechanism of BAP1 function is not yet fully clarified, further research may reveal even more therapeutic options. Thereby, BAP1 has many interaction partners as well as downstream substrates, which makes it wise to test combinations of therapeutic agents that can reverse the phenotypic effect of BAP1 protein loss.