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The Inactivation of the Gene NF2 and Its Protein Product Merlin as a Mechanism of Malignant Mesothelioma

Malignant mesothelioma is an extremely aggressive form of cancer that affects mesothelial cells. These cells act to form a slippery and protective layer of cells that line the serous cavities and internal organs of the body. Malignant mesothelioma most often develops in the pleural space, but also arises in the peritoneum, pericardium, tunica vaginalis testis, and ovarian epithelium (Vinícius and Isoldi, 2013). Evidence has shown a strong relationship between asbestos exposure and malignant mesothelioma, and this is the most widely known disease vector of malignant mesothelioma. Asbestos is a family of carcinogenic silicate fibers that are able to infiltrate and damage the pleural space of the lungs and is tightly correlated with malignant mesothelioma. However, exposure to erionite and simian virus 40 (SV40), as well as genetic predisposition, have also been associated with malignant mesothelioma (Vinícius et al., 2014). Despite these alternate agents, the leading cause of the disease remains asbestos exposure. Studies have shown that 80% of individuals diagnosed with malignant mesothelioma had previous asbestos exposure. Asbestos is a common material used in many commercial products because it is cost effective and has useful properties in many industries. The result is that despite it deadly affects, asbestos has not been banned by most countries including the U.S. and is still in wide use today. In fact, in 2003 over 2 million tons were mined and used in products worldwide (Vinícius and Isoldi, 2013).

Malignant mesothelioma displays an unusually long latency period, often up to 40 years after exposure before being diagnosed. It is believed that during this time mutations arise in key regulatory genes. This results in over 60% of malignant mesothelioma patients being diagnosed in the fifth and seventh decade of their life. Males are at a much higher risk for developing the disease, most likely because of occupational exposure (Vinícius and Isoldi, 2013). The disease is often diagnosed in the late stages and acts quickly. Prognosis is grim, and treatments are largely ineffective and have changed little over time. Median survival after diagnosis is just 11 months. Because of both continued exposure as well as the usually long latency period, the disease will remain a problem for the foreseeable future. It is expected that 70,000 new cases of malignant mesothelioma will arise over the next 20 years in the U.S. alone (Vinícius et al., 2014). While it may be difficult to prevent all asbestos exposure, studying and understanding the cellular mechanisms of this aggressive disease will aid in developing new treatments to better combat it and help thousands of patients across the world.

The mechanism by which asbestos causes malignant mesothelioma is still not fully understood, but is thought to be multifaceted. One proposed map of the pathways involved is shown in figure 1. It is known that airborne particles of asbestos become trapped inside the lungs triggering an inflammatory
response of pro-inflammatory cytokines that results in the activation of promoters of antiapoptotic genes called oncogenes (Vinícius and Isoldi, 2013). The specific pathways and implicated genes and proteins of the disease are multifaceted, not fully understood, and numerous. However, key discoveries have given some insight, and several possible insight pathways have come to light.

Generally, it has been shown that malignant mesothelioma often exhibits loss of chromosomes 1, 3, 4, 6, 9, 13, 14 and 22. One of the most common changes in diseased cells is the homozygous deletion of p14ARF and of p16INK4a genes. Both of which are involved in cell cycle regulation by regulating the G1/S checkpoint of the cell cycle. p16INK4A is a INK4 protein and has been identified as a tumor suppressor in many cancers. p14ARF protein inhibits the mdm2/p53/p21 pathway and also acts as a tumor suppressor. In contrast to this finding, and unlike many other cancers, the protein p53 encoded by TP53 has been found to have a fairly low mutation rate in the disease. This gene plays an important role in the cells response to DNA mutations. Although it has not been ruled out in malignant mesothelioma, it is not fully understood. Also, the PI3K/AKT/mTOR pathway has shown to be activated in the disease and is responsible for cell proliferation, survival, and metastasis in most cancers. In relation to this pathway, the gene PTEN has also been implicated as perhaps a pathway regulator and being altered in the disease. However, this is a new development and is ongoing. One pathway of increasing interest is the tumor suppressor Neurofibromatosis type II (NF2). Although not an initial focus of research, several recent studies have shown NF2 inactivation is a frequent event in the disease with rates ranging from 20% to 60%. NF2 is associated with the suppression of several mitogenic signaling pathways. This NF2 pathway will be the basis of investigation for this paper. Lack of expression has been previously associated with brain tumors, and other cancers. NF2 is located on chromosome 22q12, and codes for a 595 amino acid protein called Merlin (Moesin-ezrin-radixin-like protein). The first groups to look at NF2’s implication in malignant mesothelioma demonstrated that it was mutated in approximately 40–50% of cases, leading to the conclusion that its suppression was important in the tumorigenesis of the disease. Recent studies have strengthened this data. They show that 38% of pleural malignant mesothelioma samples displayed NF2 mutation, and 29.4% displayed deletions. This is in contrast to non-malignant mesothelioma lung cancer where no NF2 mutation was discovered. Other studies have found miRNA expression targeting NF2 in the disease (Vinícius et al., 2014). Although the cellular mechanism of NF2 tumor suppression is not fully understood, the protein product of the gene is structurally related to the Ezrin–Radixin–Moesin (ERM) family of proteins. These proteins are known to act as linkers between membrane and the cytoskeleton. Similar to the ERM proteins, merlin has an amino-terminal protein 4.1 family domain (FERM), as well as a long C-terminal alpha helical region. Also, the localization of merlin at areas of membrane remodeling point to functions related to the invasive properties of malignant cells as well as cell adhesion,
communication, and motility (Poulakakos et al., 2006). The implications of NF2 suppression on downstream signaling pathways that may be disrupted as a result of this inactivation are not entirely understood. However, recent work has focused on NF2 acting as a regulator to other pathways implicated in malignant mesothelioma (Vinicius et al., 2014). Such pathways include the genes,\(P53\), \(YAP1\), and \(PTEN\). This research is in its beginning stages, but has offered exciting preliminary information that NF2 could act primarily as a regulator of other oncogenes (Yokoyama et al., 2008).

One of the first steps in understanding NF2’s regulation in malignant mesothelioma was to actually determine if the gene was altered in diseased tissues and to what extent. One group collected patient tissue samples from 44 malignant mesothelioma cases in order to better understand the relationship. Three asbestosis patients’ and six normal pleura patients’ tissues were also examined as a control. Tissue samples were cultured and analyzed in terms of the characteristics of NF2. The researchers accomplished this primarily by means of PCR and western blot analysis (Thurneysena et al., 2009). RNA was extracted from the cells using a standard RNA extraction kit. From this RNA, reverse transcription was performed on 400–500 ng RNA. Full length NF2 cDNA was amplified using PCR and primers. Further PCR was performed on the full length NF2 cDNA. Truncated NF2 transcripts were found in 20.5% of malignant pleural mesothelioma samples. Knowing that some samples may not contain detectable changes in the size of the NF2 transcript, and that point mutations could still be present and affect protein expression, the researchers decided to further investigate characteristics of NF2’s protein product merlin in the samples. To do this, they performed western blots on the cell cultures and found that 43% of the malignant mesothelioma cultures showed an absence of merlin (Thurneysena et al., 2009).

With these simple experiments, researchers found that the product of the NF2 gene merlin is either absent or inactive in a large percentage of cultures obtained from mesothelioma patients. The overall frequency of NF2 alteration at either the mRNA or protein level was 43%. These two findings were significant because they established a high level of NF2 alteration in malignant mesothelioma tissue samples and established a correlation between the two. Critics may point out that only 43% of samples had detectable alterations of merlin. However, this number is significant in a disease such as malignant mesothelioma where the pathways are multifaceted and not necessarily exclusive. Also, the researchers pointed out that merlin can be phosphorylated on Ser518, which causes functional inactivation. Therefore, they propose that further research will likely reveal that functional disruption of NF2 signaling is present in all mesothelioma cells (Thurneysena et al., 2009).

A second research team took the experimentation on NF2 to the next level by re-expressing the gene in malignant mesothelioma cells and observing the effects on the cells’ tumor like properties.
Knowing that malignant mesothelioma is a highly invasive cancer and massive local spreading is common, they chose to compare local spreading (wound healing) in cells from two malignant mesothelioma cell lines (Meso17, Meso25) that either had non-functional NF2 or had been restored to have functional NF2. An adenovirus construct that expresses NF2-518A and green fluorescent protein (GFP) transfect cells and restore NF2. The GFP ensured the re-introduction of active merlin into a high percentage of diseased cells after it was shown to be expressed in more than 95% of the cells. A control of NF2-deficient malignant mesothelioma cells infected with empty adenoviral vector was also performed. The assay was monitored by time-lapse videomicroscopy as cells were cultured and grown to confluency. After infection with the virus, a wound channel was made down the center of each well with a sterile pipette. Time-lapse images were captured at 10 minute intervals over a 24 hour period and temperature was controlled at 37°C. The surface area covered by the cells as well as velocity of cell spreading was then estimated. They found that re-expression of merlin resulted in a marked decrease in the motility of the cells into the wound area of both Meso17 and Meso25 cells compared with the control infected cells. Although these cells were still technically a malignant mesothelioma cell line, their tumor like spreading properties showed a marked decrease. Specifically, cells expressing functional merlin covered about half of the empty surface area that was covered by the control cells that had non-functional merlin. It was also discovered that cells with re-expressed functional merlin consistently moved with less velocity than cells with no functional NF2 or merlin. Data and images displaying this finding are shown in Figure 2 (Poulikakos et al. 2006).

Increased invasiveness and cell motility in vitro is known to correlate with greater malignant and invasive properties in vivo. Since both the area infiltrated and the velocity of movement were greater in cells that had non-functional NF2 than cells where NF2 was re-expressed, the experiment provides clear evidence that NF2 and its protein product have a direct effect on the tumor-like properties of malignant mesothelioma. This suggests that merlin either performs independent functions of tumor suppression, or regulates a pathway that in turn imparts tumor suppression. This finding is significant in that it demonstrates a link between the presence of functional NF2 and merlin and tumor like properties of malignant mesothelioma cells. This is an important implication of a model of malignant mesothelioma which involves the loss or suppression of NF2 and merlin resulting in or at least contributing to the tumor like properties of diseased cells in malignant mesothelioma (Poulikakos et al. 2006).

Although evidence clearly demonstrates that NF2 is modified in malignant mesothelioma, a third research team desired to uncover the actual mechanism in which NF2 was inducing or contributing to malignant mesothelioma. The pathway is complex and not fully understood. However, they hypothesized that NF2’s protein product, merlin, acted as a negative regulator of YAP1. YAP1 is a protein encoded by
The YAP1 oncogene that has been shown to play a positive role in other malignant cancers. It acts as a transcriptional co-activator that upregulates genes that promote cell growth while also inhibiting apoptosis. The study first showed that downregulation of YAP1 inhibited mesothelial cell proliferation, whereas upregulation YAP1 induced mesothelial cell proliferation. This demonstrated a role of YAP1 in malignant mesothelioma. To test the hypothesis that merlin acted as a negative effector of YAP1, researchers cotransfected both YAP1 and NF2 expression vectors into a malignant mesothelioma cell line with a prior deletion of the NF2 gene. They then used immunoprecipitation to look at whether exogenous merlin has an effect on the phosphorylation status of YAP1 by using an antibody against phosphorylated serine 127 of YAP1. This site was chosen because it is a critical phosphorylation site that has been shown to cause inactivation of YAP1 as a transcription coactivator. The data collected from the experiment demonstrated that cotransfection with both YAP1 and NF2 expression vectors clearly induced the phosphorylation of YAP1 at S 127 (Yokoyama et al., 2008) (figure 3 A and B).

The implications of this experiment were important because it was one of the first studies that aimed to uncover the cellular mechanism by which NF2 acts to induce malignant mesothelioma. Other studies were important in establishing NF2 as one of many genes that may be involved in the disease, but the true mechanism of action remained a mystery. Yokoyama et al. were among the first to propose a possible mechanism based on the results of this experiment. The data clearly indicates that Merlin-dependent phosphorylation inhibits the nuclear localization of YAP1, which might result in inactivation of YAP1 transcriptional activity. Merlin does not act as a kinase and directly phosphorylate Yap1, but rather is thought to act by regulating the hippo signaling pathway which ultimately results in phosphorylation. This finding, when compounded with the knowledge that that activation of YAP1 induced mesothelial cell proliferation, results in support for a mechanism of action for merlin. In this mechanism merlin acts as a pathway regulator that goes awry to induce or at least contribute to malignant mesothelioma. Specifically, the model proposes merlin causes phosphorylation of YAP1 at the S 127 site which results in its inactivation. Since YAP1 is a known malignant tumor activator, it reasons that merlin, which is lost with the alteration of NF2, acts to suppress the gene and inhibit malignant cell proliferation (or conversely permit malignant cell proliferation when absent). This was an important finding and sheds light on one possible mechanism in which NF2 acts in malignant mesothelioma (Yokoyama et al., 2008).

The research presented has demonstrated a clear link between NF2 alteration and malignant mesothelioma. The experiments have shown that NF2 and merlin are inactivated or suppressed in a large percentage of malignant mesothelioma tissue cultures. Furthermore, it has been shown that re-expression of NF2 in mesothelioma cell lines has resulted in a retraction of some tumor-like characteristics. Although the mechanism of action of NF2 in relation to the gene remains complex, there is evidence that it at least
partially acts by regulating the expression of the YAP1 gene product. These relatively recent findings bring optimism into potential new malignant mesothelioma treatments. Treatment options for the disease have remained relatively unchanged for many years, and the prognosis is grim for this very aggressive form of cancer. This combined with the continued use of asbestos results in a true need for new and effective treatments. NF2 appears to be one of many genes involved with the disease that could offer a target for treatment of this deadly disease. If NF2 suppression plays a role in causing the tumors, then it stands to reason that somehow turning the gene back on could result in an effective treatment. I believe that ample evidence has been uncovered showing NF2’s role in the disease and that re-expression can result in reversal of tumor-like properties. With this, I believe that research should focus on methods of re-expressing the gene and the subsequent effects on malignant mesothelioma in an animal model. Perhaps gene therapy using transfection or even the development of a new drug could re-activate NF2 and put its tumor fighting properties back to use. At a minimum, the data offers enough for further investigation of the pathway. Of further interest is research that was done at The University of Montana on the effect of NF2 as a regulator of a separate pathway involving P53 and PTEN. However, this research is young and needs to be explored further. What is clear at the present moment is that NF2 and its protein product play an important role in mechanism of malignant mesothelioma and are deserving of further investigation as a potential therapeutic target.
Figures

Figure 1. A proposed pathway map of malignant mesothelioma. (Vinicius et al. 2014)

Figure 2. Re-expression of merlin on malignant mesothelioma cell motility. (Poulikakos et al. 2006)
Figure 3. Functional Interaction between YAP1 and merlin. (Yokoyama et al. 2008)
References


Pub Med Searches

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1. The function, mechanisms, and role of the genes PTEN and TP53 and the effects of abiraterone in the development of malignant mesothelioma: a review focused on the genes' molecular mechanisms.

2. Primary intrathoracic malignant mesothelioma with multiple lymphadenopathies due to non-tuberculous mycobacteria: a case report and review of the literature.

3. (Peripheral Malignant Mesothelioma: Review and recent data).

4. Malignant mesothelioma: new insights into a rare disease.

5. The role of key genes and pathways involved in the tumorigenesis of Malignant Mesothelioma.

6. Role of microRNAs in malignant mesothelioma.

7. The diagnosis of (thoracic malignant mesothelioma: practical considerations and recent}

8. (Malignant Mesothelioma: Treatment and Prognosis).

9. Malignant mesothelioma: new insights into a rare disease.

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Review on clinical trials of targeted treatments in malignant mesothelioma.


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Histone deacetylase inhibitors as potential therapeutic agents for the treatment of malignant mesothelioma.


Molecular pathogenesis of malignant mesothelioma.


Effectiveness of mesothelin family proteins and cytoreduction for malignant mesothelioma.


A potential therapeutic strategy for malignant mesothelioma with gene medicine.
Ranpirnase—an antitumour ribonuclease: its potential role in malignant mesothelioma.

117. Pan lum CY, Vogelzang NJ.
PUBMED: 15048795 [PubMed - indexed for MEDLINE]
Related citations

Recognition of histopathologic patterns of diffuse malignant mesothelioma in differential diagnosis

PUBMED: 16351662 [PubMed - indexed for MEDLINE]
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Malignant mesothelioma: current status of histopathologic diagnosis and molecular profile.

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Related citations

Malignant mesothelioma.

PUBMED: 16001964 [PubMed - indexed for MEDLINE]
Related citations

Chemotherapy for malignant mesothelioma.

PUBMED: 15955503 [PubMed - indexed for MEDLINE]
Related citations

[Development in the treatment of malignant mesothelioma.]

PUBMED: 15855965 [PubMed - indexed for MEDLINE]
Related citations

Pneurocytoma and decortication for malignant mesothelioma.

PUBMED: 25502860 [PubMed - indexed for MEDLINE]
Related citations

The emerging role of pemetrexed for the treatment of malignant mesothelioma.

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Malignant mesothelioma—the UK experience.

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Related citations

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PUBMED: 15351447 [PubMed - indexed for MEDLINE]
Related citations
Moving beyond chemotherapy: novel cytostatic agents for malignant mesothelioma.

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The use of magnetic resonance imaging in malignant mesothelioma.

PMID: 15291608 [PubMed] [Indexed for MEDLINE]

Clinical-pathological and biological prognostic factors in pleural malignant mesothelioma.

PMID: 15291609 [PubMed] [Indexed for MEDLINE]

Pathology of malignant mesothelioma.

PMID: 15291610 [PubMed] [Indexed for MEDLINE]

Experimental therapy of malignant mesothelioma: new perspectives from anti-angiogenic treatments.

PMID: 15167798 [PubMed] [Indexed for MEDLINE]
Results: 17

1. LIM-domain protein AURKA suppresses malignant mesothelioma cell proliferation via Hippo signaling cascade.
   Oncogene. 2012 Dec 10; doi: 10.1038/onc.2012.523. [Epub ahead of print]
   PMID: 23485320 [PubMed - indexed by publisher]
   Related citations

2. The role of key genes and pathways involved in the tumorigenesis of Malignant Mesothelioma.
   de Andrade L.V, Locali M, Ida RK, Rimaudo MD.
   PMID: 24941441 [PubMed - indexed by publisher]
   Related citations

3. YAP induces malignant mesothelioma cell proliferation by upregulating transcription of cell cycle-promoting genes.
   PMID: 22586194 [PubMed - indexed for MEDLINE]
   Related citations

   Sekido Y, Carcangiu M.
   PMID: 23777860 [PubMed - indexed for MEDLINE]
   Related citations

5. Convergent signaling in the regulation of connective tissue growth factor in malignant mesothelioma. TGFβ signaling and defects in the Hippo signaling cascade.
   Fujii M, Nakamura M, Toyoda T, Tamaki I, Kondo Y, Osada H, Sekido Y.
   Related citations

6. TGF-β synergizes with defects in the Hippo pathway to stimulate human malignant mesothelioma growth.
   Related citations

   Sekido Y.
   PMID: 21655993 [PubMed - indexed for MEDLINE]
LAT52 is a tumor suppressor gene in malignant mesothelioma


Chemotherapeutic receptor tyrosine kinases in malignant mesothelioma as a rationale for combination therapy.


Genomic abnormalities and signal transduction dysregulation in malignant mesothelioma with.

Sekido Y.

CDK6, NFI2, and JUN are dysregulated among other genes by miRNAs in malignant mesothelioma.


A conditional mouse model for malignant mesothelioma.


Neuroendocrine tumors of the NS (NF2) and malignant mesothelioma in a man with a constitutional NF2.

Mossman BM, Uy H, Wallace AJ, Evans D.D.

Molecular biology of malignant mesothelioma.

Sekido Y.

Animal models of malignant mesothelioma.

Kato A.B.

Cytogenetic and molecular genetic changes in malignant mesothelioma.
