From the Department of Laboratory Medicine
Karolinska Institutet, Stockholm, Sweden

EXPERIMENTAL THERAPEUTICS AGAINST MALIGNANT MESOTHELIOMA – INVESTIGATIONS *IN VITRO*

Gustav Nilsonne, M.D.

Karolinska Institutet

Stockholm 2009

"Nullius addictus jurare in verba magistri"

Horatius

Front cover: *Amazon and Centaur*, by Franz von Stuck. **Back cover:** *Spear-throwing Amazon* and *Wounded Centaur*, by Franz von Stuck.

The centaur is a violent and brutal creature of a double nature, seen on the front cover assaulting with a lump-shaped weapon (lat. *tumor*). The amazon is a fierce fighter armed in this picture with a spear, i.e. a targeted intervention. On the back cover, the outcome of the battle is evident.

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet. Printed by ABA Kopiering.

ISBN 978-91-7409-734-4

To my family
Åsa
Ulf
Carl
Hjalmar

ABSTRACT

Malignant mesothelioma is a tumour of the mesothelium, a specialized tissue found in the pleura, pericardium, peritoneum, and tunica vaginalis testis. The tumour has strong etiological links to asbestos, with a latency period ranging up to several decades between asbestos exposure and tumour diagnosis. Treatment is mostly ineffective and the median survival is around 12 months.

The tumour exhibits an interesting and peculiar heterogeneity; tumour cells may assume either an epithelioid or a sarcomatoid phenotype. Presence of the sarcomatoid phenotype is a predictor for therapy resistance and a worse prognosis.

In this work, we have investigated the anticancer effects of selenite, a small ion formed by the oxidation of selenium. We show that selenite can induce cell death and apoptosis in malignant mesothelioma cells. The specific signalling pathways have been delineated. Furthermore, we have shown that selenite sensitizes mesothelioma cells to NK-cell mediated recognition and killing.

Specifically, we have shown that selenite exerts its anticancer effects in cells of both phenotypes through the induction of oxidative stress. Apoptosis is induced through the mitochondrial pathway. Differences in the activation pattern of Bcl-family proteins could be observed between cells of the two phenotypes. Interestingly, p53 protein was enriched in the nuclei of selenite-treated cells, but in a form bereft of its DNA-binding ability. We hypothesise that this is explained by redox inactivation due to the prooxidant effects of selenite. Perturbation of the apoptosis signalling network by inhibition of key mediators had very little effect, suggesting that the network is robust through functional redundancy.

A systematic testing of selenite in a panel of six cell lines of varying differentiation alone and together with conventional drugs showed that the cells were resistant in general, but all cell lines were sensitive to at least one of the tested drugs. Synergistic effects between selenite and other drugs were limited. Phenotype was a poor predictor of response. These results highlight the need for improved personalisation of mesothelioma treatment.

In the last sub-project, we have showed that selenite sensitizes mesothelioma cells to NK-cell mediated killing. A search for alterations in NK-cell receptor ligand expression revealed that the inhibitory ligand HLA-E was down-regulated on tumour cells in response to selenite. Further analyses showed that the mRNA expression for HLA-E remained constant during selenite treatment, while the intracellular and surface protein levels decreased, and that increased recognition by NK cells was dependent on HLA-E downregulation rather that on modulation of other NK cell ligands.

These results indicate that selenite is a potential new drug against malignant mesothelioma. Further trials are warranted to determine the safety and efficacy of selenite in a clinical setting.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Malignt mesoteliom är en ovanlig tumör som orsakas av asbest. Den uppstår oftast i lungsäcken, men kan också förekomma i bukhålan och hjärtsäcken. Trots att skadeverkningarna av asbest har varit kända sedan 1960-talet har antalet nyupptäckta mesoteliomfall per år inte börjat minska. Detta beror främst på att det kan gå 3-4 decennier mellan exponering och diagnos. Malignt mesoteliom är svårt att diagnostisera och mycket svårt att behandla. Medianöverlevnadstiden från diagnos är ungefär ett år, och runt 10% av drabbade är vid liv fem år från diagnostillfället. Dessa tal har inte förbättrats nämnvärt under de senaste tjugo åren.

Detta arbete handlar huvudsakligen om ett experimentellt läkemedel: selenit. Effekten av selenit har undersökts på tumörceller i odling. Vi visar att selenit orsakar programmerad celldöd, s.k. apoptos, i mesoteliomceller. Vi visar också att mesoteliomceller är känsligare för selenit än normala celler från lungsäcken, som inte är tumöromvandlade. Selenitens effekt beror på att den orsakar oxidativ stress, dvs produktion av reaktiva syremolekyler som reagerar med cellens olika beståndsdelar så att dessa förlorar sin normala fuktion.

Närmare undersökningar av hur selenit orsakar apoptos har bland annat visat att selenit leder till produktion av proteinet p53, som har kallats ”celldödens dirigent”, och att p53 förflyttas till cellkärnan, där det normalt styr uttrycket av en mängd gener. Dock ansamlas p53 i cellkärnan i en inaktiv form, som saknar förmåga att styra celldödsprocessen. Vi har föreslagit förklaringen att det är den oxidativa stress som följer av selenitbehandling som leder till inaktiveringen av p53.

Jämförelser mellan selenit och konventionella cytostatika visar att selenit är mera effektivt i koncentrationer som teoretiskt skulle kunna ges till patienter. Sex olika mesoteliomcellinjer ingick i dessa försök. Samtliga var generellt högt motståndskraftiga mot cytostatika, men samtidigt visade alla sex känslighet för något eller några av de testade läkemedlen, dock sällan samma. Dessa resultat tyder på att ökad individanpassning av läkemedelsbehandling vid mesoteliom kan leda till bättre behandlingseffekt.

Vidare har vi undersökt hur selenit påverkar immunförsvarets igenkänning av tumörceller, och i synnerhet den s.k. NK-cellen, som är den komponent av immunförsvaret som framför allt har till uppgift att upptäcka och döda celler som är skadliga för organismen till följd av tumöromvandling eller virusinfektion. Selenit ökar igenkänningen av mesoteliomceller, och vi har också kunnat visa att detta beror på en minskning av molekylen HLA-E på tumörcellens yta, vilken i vanliga fall hämmar NK-cellsaktivering.

Dessa fynd talar sammantaget för att selenit skulle kunna användas som ett nytt läkemedel mot malignt mesoteliom. Ytterligare studier krävs för att utvärdera säkerheten och den eventuella behandlingseffekten hos patienter.

LIST OF PUBLICATIONS

I. **Selenite induces apoptosis in sarcomatoid malignant mesothelioma cells through oxidative stress.** *Free Radical Biology & Medicine (2006)* ***41****:874–885.* doi:10.1016/j.freeradbiomed.2006.04.031

Gustav Nilsonne, Xiaojuan Sun, Christina Nyström, Anna-Klara Rundlöf, Aristi P Fernandes, Mikael Björnstedt, Katalin Dobra.

II. **Phenotype-dependent apoptosis signalling in mesothelioma cells after selenite exposure.** *Journal of Experimental and Clinical Cancer Research (2009)* ***28****:92* doi: 10.1186/1756-9966-28-92

Gustav Nilsonne, Eric Olm, Adam Szulkin, Filip Mundt, Agnes Stein, Branka Kocic, Anna-Klara Rundlöf, Aristi P Fernandes, Mikael Björnstedt, Katalin Dobra.

III. **Great variation in sensitivity of malignant mesothelioma cell lines to different anti-cancer drugs and their combinations highlights the need for individualized therapy.** *Submitted.*

Adam Szulkin, **Gustav Nilsonne**, Filip Mundt, Pegah Souri, Anders Hjerpe, Katalin Dobra.

IV. **Oxidative Stress-Induced Downmodulation of HLA-E Sensitizes Tumour Cells to killing by CD94/NKG2A-expressing NK Cells.** *Manuscript.*

Mattias Carlsten, Monika Simonsson, **Gustav Nilsonne**, Oscar Hammarfjord, Niklas Björkström, Mikael Björnstedt, Anders Hjerpe, Hans-Gustaf Ljunggren, Katalin Dobra, Karl-Johan Malmberg.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CEA	Carcinoembryonic Antigen
CK-HMW	High Molecular Weight Cytokeratin
CT	Computed Tomography
DCF	Dichlorofluorescein
DNAM-1	DNAX-accessory molecule 1
DTNB	5,5'-Dithiobis-(2-nitrobenzoic acid)
EGF	Epidermal Growth Factor
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	Epithelial Membrane Antigen
FISH	Fluorescent In Situ Hybridisation
FGF	Fibroblast Growth Factor
GSH	Glutathione (reduced)
HGF	Hepatocyte Growth Factor
HLA	Human Leukocyte Antigen
IAP	Inhibitor of Apoptosis
JNK	c-Jun N-terminal Kinase
KIR	Killer Ig-like Receptor
LIR	Leukocyte Ig-like receptor
MDM2	Murine Double Minute homologue-2
MHC	Major Histocompatibility Complex
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NCR	Natural Cytotoxicity Receptor
NK cell	Natural Killer cell
PDGF	Platelet-Derived Growth Factor
RSH	Thiol group on protein or peptide (reduced)
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SeCys	Selenocysteine
SeMet	Selenomethionine
SCID	Severe Combined Immunodeficiency
SV40	Simian Virus 40
TNF-α	Tumour Necrosis Factor-α
TrxR	Thioredoxin Reductase
WBC	White Blood Cell count
WST-1	Water Soluble Tetrazolium-1
XIAP	X-linked Inhibitor of Apoptosis Protein

1 BACKGROUND

1.1 BIOLOGY OF THE PLEURA AND MESOTHELIUM

1.1.1 Structure and function of the mesothelium

The mesothelium is the specialized lining of the serious cavities, comprising the pleura, peritoneum, pericardium, and tunica vaginalis testis. Several functions have been ascribed to the mesothelium [1]:

1. To facilitate movement with a minimum of friction against the visceral organs that are covered by the mesothelium
2. To regulate transport of proteins across the serosal surfaces
3. To regulate inflammatory and regenerative processes including the migration of inflammatory cells and the lysis of fibrin depositions

Histologically, the mesothelium has a surface layer of flattened epithelioid cells arranged in a single layer with polygonal shape and numerous microvilli found on the apical side. Beneath a thin basement membrane, there are fibroblast-like submesothelial cells with a spindle-shaped morphology.

To facilitate frictionless movement, the mesothelial cells are coated with glycosaminoglycans, particularly hyaluronan [2]. These molecules bind water and give the surface a slithery quality [3, 4]. The hyaluronan also inhibits inappropriate adhesion of cells to the mesothelial surface, thereby counteracting metastatic tumour implantation [5, 6].

Mesothelial cells often have micropinocytic vesicles, which are involved in transport of fluid and solutes across the serosal membrane [7-9]. Experimental work has shown that particles up to 100 nm size can be transported across the mesothelium in this manner [10-12]. There are also stomata ranging up to about 12 μm diameter in the mesothelial surface, first described by von Recklinghausen in 1863 [13], feeding into the lymphatic network. These stomata thus provide a means for clearance of excess fluid as well as transport of macromolecules and migrating cells from the serosal cavities [14-17].

In injury and regeneration, mesothelial cells are capable of secreting a number of cytokines, chemokines, and growth factors, including interleukins 1, 6, and 8, transforming growth factor-β (TGF-β), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) [18-26]. Some evidence indicates that mesothelial cells may perform functions of the innate immune response, such as phagocytosing bacteria, attracting and activating circulating neutrophils and monocytes, and presenting antigens [22, 27-29].

1.1.2 Epithelial-mesenchymal transdifferentiation

The histological classification of the mesothelium has been debated over the years. The mesothelium is derived from the embryonic mesoderm, and therefore a histogenetic classification will define it as a connective tissue. Following this traditional classification, malignant mesothelioma is usually defined as a sarcoma [30]. A more

functional classification based on the histomorphological characteristics of the tissue will, on the other hand, tend to group the mesothelium among the true epithelia. In modern histology, a classification based on the expression of surface adhesion molecules is often employed. Within this approach, cells expressing E-cadherin are considered epithelial, whereas cells expressing N-cadherin are considered mesenchymal [31]. Mesothelial cells are able to express both, although N-cadherin is predominant [32].

There is by now a wealth of data supporting the notion that mesothelial cells are capable of transdifferentiating along an epithelial-mesenchymal axis. The first such evidence was obtained from early studies of mesothelial regeneration. Clarke (1915) observed the formation of mesothelium around a foreign body of celloidin implanted subcutaneously in the abdomen of a dog, thus suggesting that mesothelium may form through other processes than the division of fully differentiated surface mesothelial cells [33]. Clarke's identification of the tissue as mesothelium was, however, based only on microscopic examination including silver staining and we cannot be certain that the tissue he observed was really mesothelium. Nevertheless, this study paved the way for others. Lewis (1923) observed transdifferentiation of mesothelial cells in chick embryo heart tissue cultures, both from an epithelioid to a multipolar phenotype with spiky filopodia, and in the reverse direction [34].

Maximow (1927) observed rabbit peritoneal tissue preparations after the induction of injury, and noted: "Die Mesothelzellen können sich sofort zu regelrechten, mit Ausläufern versehenen Fibroblasten entwickeln," meaning that the surface mesothelial cells in his cultures had the capacity to differentiate into a fibroblast-like morphology [35]. Stout and Murray (1942) report a case of malignant mesothelioma where tumour cells were investigated by tissue culture, and were found to transdifferentiate from an epithelioid to a sarcomatoid phenotype *in vitro*[1] [36].

After the advent of electron microscopy, Raftery (1973) studied mesothelial regeneration and was able to show that fibroblast-like mesothelial cells underlying a wounded area of mesothelium proliferate, migrate, and transdifferentiate into surface mesothelial cells to restore the mesothelial surface [37, 38]. Bolen et al. [39] studied mesothelial tissue from a series of cases of mesothelial injury and metastatic cancer, and found that the fibroblast-like mesothelial cells express high levels of vimentin, a cytoskeletal protein characteristic for mesenchymal cells, in their resting state, but begin to express cytokeratins, characteristic for epithelial cells, in response to injury, while vimentin expression is lost.

Wound healing on the serosal surface occurs not only by cell proliferation and migration from the edges of the wound (as is usual in epithelia), but also through the

[1] The authors argue, partly based on this differentiation potentiality, that the tumour was in fact a malignant mesothelioma. The entity of malignant mesothelioma was controversial at the time, and the head pathologist at the authors' department, Dr A. M. Pappenheimer, objected so strongly to this interpretation that the paper was retracted and then, when eventually re-published, carried an appendix where Dr Pappenheimer argues that the tumour was a leiomyosarcoma. He may of course have been correct; modern diagnostic methods would have settled the issue.

recruitment of distant mesothelial cells, which detach into the serosal fluid, move to the site of the injury and settle there [40]. During this process, the mesothelial cells assume a more mesenchymal phenotype with the upregulation of α_2 integrin, downregulation of cytokeratins and E-cadherin, and concomitant changes in morphology [41, 42]. This process is driven by microenvironmental cues from macrophages that aggregate around the site of injury and secrete factors such as FGF-2, TNF-α, and PDGF [43, 44]. There is also, as already mentioned, evidence that the subserosal fibroblast-like cells contribute to wound healing through migration, proliferation, and epithelial transdifferentiation [37, 39]. The relative importance of this mechanism has been questioned [45], but its occurrence is not disputed.

Interestingly, detachment and reimplantation of mesothelial cells is also the mechanism of formation for the visceral pericardium during embryogenesis. The parietal pericardium buds off cell clusters into the pericardial fluid, and these cells colonise the outer surface of the heart until the pericardium is continuous [46].

Thus, the mesothelial cell moves on a continuum of differentiation between a classical epithelial and a classical mesenchymal phenotype. It may be said to instantiate both epithelial-to-mesenchymal transition and mesenchymal-to-epithelial transition under physiological conditions. For this reason, mesothelial cells and malignant mesothelioma cells are referred to in this work as "epithelioid" and "sarcomatoid" rather than "epithelial" and "sarcomatous."

1.1.3 Do we need the pleural cavity?

The pleural cavity is the site of numerous illnesses. Apart from malignant mesothelioma, which is uncommon, the pleural cavity can be afflicted by pneumothorax, infections, effusions of lymph and blood, and autoimmune inflammation. Patients in whom the pleural cavity has been obliterated usually retain adequate pulmonary function. This has led some investigators to question whether the pleura is useful to us, or, in terms of evolutionary biology, whether its presence offers a fitness advantage.

As the pleura consists of soft tissue, the fossil record can tell us nothing about its evolution. Studies in comparative anatomy have hitherto found only one mammal that lacks pleural cavities, namely the elephant [47]. In elephants, pleural cavities are formed early in embryogenesis [48], but are later in gestation replaced by elastic connective tissue [49]. West (2001) has convincingly argued that this is likely a functional adaptation to the high pressure gradients that occur when elephants snorkel underwater, using their trunks to breathe [50, 51]. In these situations, the arterial blood pressure must exceed the water pressure to allow tissue perfusion, while the pleural cavities must maintain a negative pressure at inspiration. Therefore, small blood vessels in the pleura would be at risk of profuse transudation or even rupture.

The fitness advantage resulting from the existence of the pleural cavity is probably due to the improved ventilation of the lung. Thanks to the pleural cavity, the lung can expand efficiently to fill the pleural space, regardless whether the expansion is caused by movement of the ribs or the diaphragm. If one part of the lung should become

consolidated or atelectatic, other parts of the lung can compensate by increasing their ventilation without the necessity for any alteration of breathing dynamics.

The ubiquitous presence of the pleural cavities among mammals except the elephants suggests that they, on balance, do not carry any strong fitness penalty.

1.2 MALIGNANT MESOTHELIOMA

1.2.1 Etiology and epidemiology

The history of the mesothelioma epidemic is the history of the world's greatest industrial-injury incident – one which still goes on to this day. Malignant mesothelioma is caused by exposure to asbestos, a fibrous mineral that has been used for thousands of years due to its heat-resistant properties. The oldest archaeological finds of asbestos are from Nordic countries, where asbestos pottery was used already around 4000 BC [52]. In antiquity, asbestos was well known, and used for lamp-wicks, tablecloths, cremation shrouds, and other purposes. Records of investigations into the properties of asbestos have been left by scientists in the first century AD such as Pedanius Dioscorides and Pliny the elder [53, 54].

Industrial use of asbestos on a greater scale began in the second half of the 19th century, as development of the steam engine caused an increased demand for asbestos as an insulation material [55]. Thereafter, asbestos production increased until the 1970's, and the material found use in many applications; buildings, ships, brake linings, protective clothing, gaskets, and much else.

Asbestos exists in six different fiber types: chrysotile, actinolite, tremolite, anthophyllite, amosite, and crocidolite. Chrysotile (white asbestos) is classified as a serpentine fiber type, while the other five are amphibole fiber types. Crocidolite is also known as "blue asbestos" and is the most carcinogenic. This fiber type has only been industrially mined in Australia (Wittenoom), and in South Africa [56]. Amosite is known as "brown asbestos" and derives its name from "Asbestos Minerals of South Africa".

Asbestos exposure has varied greatly between geographic locations, and present incidence rates of malignant mesothelioma correspond to historic exposure. Areas of high incidence are found around industries involved in extraction and processing of asbestos. Examples include asbestos mines, shipyards, and insulation manufacturers.

In Central Anatolia in Turkey, there is an area where an asbestos-like mineral called erionite is prevalent in the topsoil. This environmental exposure, starting at an early age, leads to an extremely high local mesothelioma incidence – as much as 30% of the population may expect to develop mesothelioma during the course of their lives [57, 58].

Malignant mesothelioma was recognised by some as a tumour entity already in the early 1900's [59]. Others, however, argued that the rare cases observed could well have other histogenetic origins. Only with the discovery in 1960 of the strong etiological link between asbestos and mesothelioma was the diagnosis finally accepted [60, 61].

The risk of mesothelioma is mainly influenced by exposure to asbestos, in a dose-dependent relationship [62]. The main risk groups are workers in asbestos mining and processing, shipyards, and construction, and their families through secondary exposure [52]. Interestingly, almost no other risk factors due to exposure have been found to be important – not even smoking [63]. A causative role for Simian Virus 40 (SV40) has previously been suggested based on the detection of viral DNA in tumour tissue samples and of viral protein by immunohistochemistry [64, 65]. However, later work has cast doubts on this hypothesis, because many case series from outside the United States have been uniformly negative or shown SV40 prevalence rates consistent with expected rates in the general population [66-70].

The incidence of malignant mesothelioma depends heavily on the diagnostic methods used, as many mesotheliomas can only with difficulty be differentiated from adenocarcinomas metastatic to the pleura with conventional histopathology [71]. Figure 1 shows the incidence of malignant mesothelioma in Sweden from 1970 to 2006, as reported to the National Cancer Registry. Much of the increase between 1970 and 1990 is due to increased accuracy of diagnosis. The figure also illustrates that the incidence is considerably higher for men than for women, a relationship that is determined by the occupational exposure to asbestos in professions dominated by men.

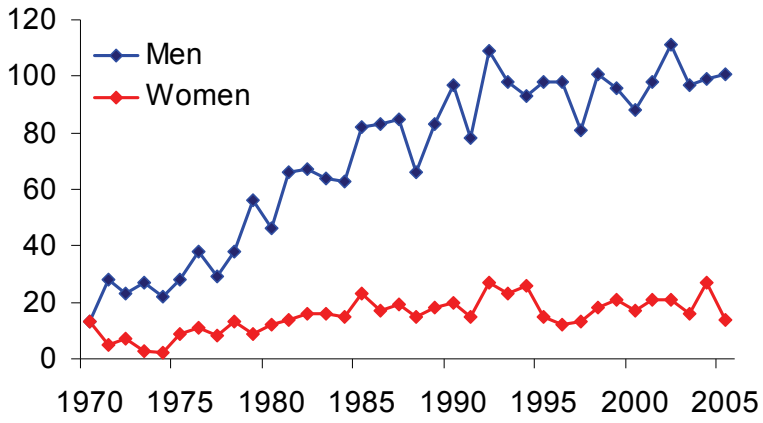

Figure 1. Incidence of mesothelioma in Sweden 1970-2005. Data from the Swedish National Cancer Registry.

Although crocidolite is no longer mined, the production of chrysotile asbestos is larger than ever, taking place mainly in Russia, Canada, China, and Kazakhstan [72, 73]. The primary markets are China, India, Brazil, Russia, Thailand, and Japan, where an increase in malignant mesothelioma may consequently be anticipated over the coming decades. The World Health Organisation estimates that 5-10 million people will die from asbestos-related disease, including mesothelioma, lung cancer, and asbestosis [72].

1.2.2 Pathogenesis

The mechanism by which asbestos induces malignant mesothelioma has not been fully elucidated. Asbestos was thought for a long time to be biologically inert, and its carcinogenic effects were therefore difficult to explain. One proposed mechanism was entirely biomechanical: it was suggested that the spiky end of an asbestos fiber might

interfere with the microtubules of the mitotic spindle apparatus, leading to chromosomal imbalances in the daughter cells [74].

It is now known that asbestos fibers cause oxidative stress in the tissue where they are embedded, due to the catalysis of free radicals by iron in the fibers [75, 76]. This stress is likely to cause a permanent process of tissue injury and regeneration. Indeed, asbestos-induced pleuritis can be seen in patients with heavy asbestos exposure, and is considered by some to represent a pre-malignant stage of asbestos disease [77, 78]. Mesothelial cells and macrophages exposed to asbestos during in vitro culture secrete considerable amounts of growth factors, such as TNF-α, TGF-β, and PDGF, indicating a regenerative response to tissue injury [79].

The so-called "overhealing wound" hypothesis is an old explanatory model for carcinogenesis [80]. In wound healing, a local signalling environment is formed that strongly promotes proliferation, invasion and differentiation, through the secretion of specific growth factors including members of the epidermal growth factor (EGF), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) families [80]. Local stromal cells such as myofibroblasts and macrophages are important effector cells in this signalling, and their activity has many similarities in wound healing and cancer [80]. A particularly interesting observation in this context is the tropism of many mesotheliomas towards injured areas. In particular, implantation metastases may often be observed in wound canals from thoracocentesis or thoracoscopy [81].

Many malignant mesotheliomas share certain cytogenetic changes. Deletions of the short arms of chromosomes 1, 3, and 9, and the long arms of chromosome 6 and 22, are common [82, 83]. Polyploidy is not very common. In a recent case series, 21 out of 22 mesotheliomas had 40-55 chromosomes, and one had 92 [84].

Specific genetic changes often found in mesotheliomas include the inactivation of the tumour suppressor genes INK4a, encoding the p16 protein, and NF2 [83, 85-87]. p53 is seldom mutated [88-90], but may often be inactivated instead through the inactivation of INK4a, as this gene also encodes the p19 protein, which inhibits the negative regulator of p53 MDM2 (murine double minute homologue-2) [91]. Tyrosine kinase receptors including the epidermal growth factor receptor (EGFR) and c-Met are often epigenetically upregulated in mesothelioma, contributing to a proliferative drive [92, 93].

Mesothelioma cells have a marked apoptosis resistance [94]. This trait has been linked to the above-mentioned INK4a deletion, as well as high expression of the antiapoptotic Bcl-2 family members Bcl-XL and MCL1 [95], and of IAP family proteins such as XIAP, IAP1, survivin and livin [96-99].

Tumours are in general heterogeneous, as is expected from the model of tumourigenesis as an evolutionary process. According to this model, more or less random changes to the genetic and epigenetic regulation of the tumour cells will cause variation within the tumour. Cells that happen to acquire a more malignant phenotype will then have a fitness advantage and outcompete the less malignant cells, leading to

tumour progression. The fitness advantage may lie in any of the areas defined as hallmarks of cancer: proliferative drive, apoptosis resistance, limitless replicative potential, etc [100].

In malignant mesothelioma, a peculiar pattern of heterogeneity can be seen where the tumour cells can assume either an epithelioid or a sarcomatoid morphology and tissue organisation. As mentioned above, transdifferentiation on the epithelial-mesenchymal axis is a capacity of the normal mesothelium. This heterogeneity is functionally significant in mesotheliomas, as the presence of sarcomatoid cells is a strong predictor for worse prognosis and therapy resistance (see section 1.2.5).

Comparisons of the biology of epithelioid and sarcomatoid mesothelioma cells have been made with several methods. Gene expression analysis using Affymetrix chips robustly classifies epithelioid and sarcomatoid tissue samples using unsupervised hierarchical clustering [101], indicating that the differences between the cell types are not just morphological, but reflect a pervasive difference in cell function.

Most probably, mesothelioma cells retain the capacity of their benign counterparts to transdifferentiate on the epithelial-mesenchymal axis, in response to signalling from the microenvironment. Such transdifferentiation can be seen in mesothelioma cells *ex vivo* as well as in established cell lines (see section 1.1.2). The epithelioid STAV AB and the sarcomatoid STAV FCS mesothelioma cell lines have been used to establish xenograft tumours in SCID mice, and a thorough characterisation revealed that both cell lines transdifferentiated into a similar intermediate phenotype (Dárai-Ramqvist et al, unpublished).

1.2.3 Diagnosis

Patients with malignant pleural mesothelioma typically present with dyspnea as the main symptom. This is usually caused by a pleural effusion, which may require drainage. Radiological examination using computed tomography (CT) often reveals the presence of a tumour, while the diagnosis in general and the differential diagnosis between malignant mesothelioma and other malignancies in particular must be obtained from histopathological and cytological analyses.

The pleural fluid is in many cases the first material to be examined, as thoracocentesis is often performed for symptom relief. In many cases biopsies are also analysed, often taken by means of thoracoscopy. In cytology, signs of malignant mesothelioma include cell clustering in papillary structures, nuclear pleomorphism, nuclear hyperchromasia, and multinuclearity [71, 102]. In many cases it is however difficult to distinguish between mesothelioma cells and reactive mesothelial cells, which during inflammation undergo morphological changes described in section 1.1.

In histopathology, epithelioid mesotheliomas appear as atypical epithelioid cells arranged in tubules, acini, papillae, or sheets. A relatively wide range of differentiation can be observed among the epithelioid cells with different morphologies, including

small cuboidal, large columnar, macrophage-like, and anaplastic phenotypes. Sarcomatoid mesotheliomas show a dense, spindled pattern with fibroblast-like cells in a variable amount of collagenous or myxoid stroma [71].

Adjuvant technologies have in the past decades led to a markedly increased diagnostic accuracy. Immunohistochemical stainings for markers such as calretinin, mesothelin, WT-1, HBME-1, D2-40, epithelial membrane antigen (EMA), high molecular weight cytokeratin (CK-HMW), and vimentin can demonstrate the mesothelial origin of tumor cells (depending on staining pattern), whereas differentiation between mesothelioma and adenocarcinoma is aided by epithelial markers such as BerEp4, sialosyl T, carcinoembryonic antigen (CEA), and TTF-1 [103]. In electron microscopy, a finding of desmosomes, neolumina, numerous slender microvilli without rootlets, and absence of glycocalyx is pathognomonic for malignant mesothelioma. These adjuvant diagnostic measures can also be applied to cells and cell groups from effusions, often allowing an accurate diagnosis based solely on cytological material.

Of the cytogenetic techniques, it is fluorescent in situ hybridisation (FISH) that has proven to be of greatest use. As mentioned above (section 1.2.2), p16 is commonly deleted in mesotheliomas. FISH analysis for p16 deletion as well as numerical chromosomal aberrations is useful in clinical diagnosis [86, 87, 104].

In addition to these techniques for investigating the tumour cells, several soluble biomarkers are useful for diagnosis. Hyaluronan, which is secreted by normal mesothelial cells (see section 1.1), is often produced in large quantities by mesotheliomas and can be analysed in the pleural fluid [105, 106]. This biomarker is highly specific and is used in many parts of the world. More recently, mesothelin and osteopontin have also been proposed as biomarkers for mesothelioma in pleural fluid [107-110].

1.2.4 Treatment

The mainstay of treatment for malignant mesothelioma is chemotherapy. Results of chemotherapy are disappointing, however, with at most a 40% response rate and an average increase in survival time of approx. 3 months [111]. Over the past decades, treatment efficacy has increased only to a very limited extent, despite the introduction of new drugs and extensive trials to optimise treatment protocols [112].

Currently, the drug of choice is Pemetrexed, a folate inhibitor, preferentially in combination with cisplatin or carboplatin [111, 113, 114]. Results similar to those obtained with Pemetrexed have been shown with the triple combination of Carboplatin, liposomized Doxorubicin, and Gemcitabine [115]. Many of the conventional drugs used today have serious side-effects. Platinum-based drugs, for example, have a marked nephro- and ototoxicity [116, 117], and Doxorubicin is cardiotoxic. These side-effects may reduce the patients' quality of life [118], and much may be gained from the development of predictive analyses to optimise drug treatment for individual patients and prevent unnecessary treatment with ineffective drugs that have serious side-effects (see section 2.3.2).

In some centres, surgery is offered to patients with early-stage disease and adequate performance status. No randomized trials to evaluate the efficacy of surgery compared to chemotherapy alone have been undertaken. Cohort studies however suggest that mortality figures are comparable [119]. Radiotherapy may be given as an adjuvant therapy to surgery, or as a palliative intervention.

1.2.5 Prognosis

Malignant mesothelioma has a dismal prognosis. A recent meta-analysis of clinical trials published from 1970 to 2005 showed that the median survival time ranged between 4 and 17 months, with no trend of improvement over time [112].

The most reliable predictor of prognosis is the presence of tumour cells with a sarcomatoid phenotype, which correlates to a poorer prognosis [120-124]. Other prognostic factors are less well established. Tumour stage has been shown to be a significant prognostic factor in some studies but not in others [121, 123, 125-127]. Two major studies have sought to establish multiparametric prognostic models for mesothelioma. The Cancer and Leukemia Group B (CALGB) [128] developed a model incorporating the following parameters: histological type, age, performance status, white blood cell count (WBC), hemoglobin (Hb), chest pain, and weight loss. The European Organization for Research and Treatment of Cancer (EORTC) [122] instead developed a prognostic model based on WBC, performance status, certainty of diagnosis, histology, and gender.

Due to the long latency period from asbestos exposure to tumour diagnosis, and the fact that most malignant mesotheliomas are diagnosed only in a late stage, it is thought that most patients exhibit a potentially detectable malignant or premalignant condition for years prior to diagnosis. With improved methods for screening and early detection, the prognosis will be expected to improve both because the patients will have longer to live under the natural course of the disease, and because treatment may be expected to have greater effect at earlier stages.

1.3 ANTICANCER EFFECTS OF SODIUM SELENITE

1.3.1 Overview of selenium biochemistry

Selenium, discovered in 1818 by Jöns Jacob Berzelius [129], is an essential trace element because of its crucial role in selenoproteins. The chemical properties of selenium are very similar to those of sulphur, as both are members of the chalcogen group in the periodic table. Substitution of sulphur with selenium in cystein and methionine yields the two amino acids selenocysteine and selenomethionine. While only selenocysteine can be synthesised by humans, selenomethionine is produced by many plants and fungi and, when present in human cells, can accidentally become inserted in the place of methionine during protein synthesis [130]. Since the selenium atom in selenomethionine is shielded by a methyl group, selenomethionine has virtually the same chemical properties as methionine. In selenocysteine, on the other hand, the exposed selenium atom is more nucleophilic than the corresponding sulphur atom in

cystein, leading to differences in enzymatic activity when these amino acids are substituted for one another [131].

A common metabolic pathway exists for all selenium compounds (Figure 2). As this figure shows, the central intermediary is selenide. Different compounds are however converted to selenide by different enzymes, and hence the rate of selenide synthesis can vary greatly between different compounds that are added to a living system.

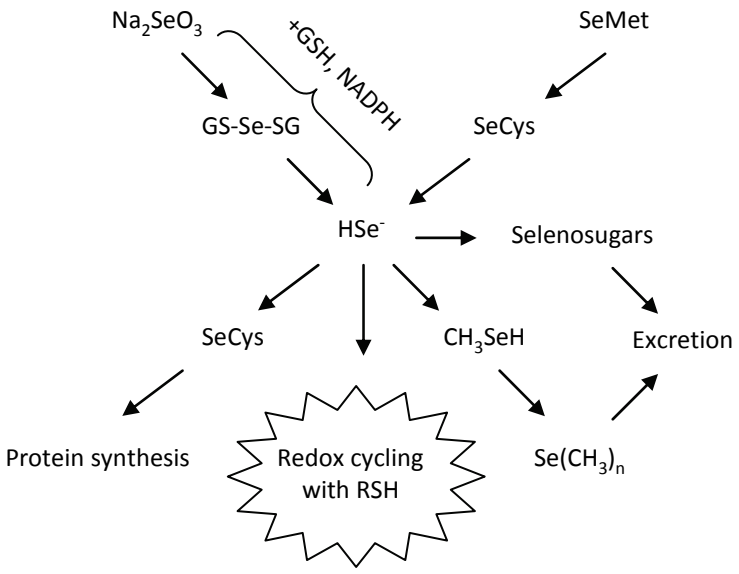

Figure 2. Simplified overview of the main pathways in selenium metabolism. Adapted from Lü and Jiang and Selenius et al [132, 133]. GSH: Glutathione (reduced). GS-Se-SG: selenodiglutathione. SeMet: Selenomethionine. SeCys: Selenocysteine. RSH: free thiols on proteins and peptides.

Selenocysteine is formed as an intermediary for the synthesis of selenoproteins, whereas methylated selenium compounds are exreted. Dimethylselenide is excreted by the breath (causing a peculiar garlic odour), and trimethylselenonium through the urine [133-135]. In addition, selenide can be metabolised into selenosugars, which are also excreted [133].

There are 25 human selenoproteins [136]. Most of these proteins are redox-active enzymes; glutathione peroxidase 1-6, thioredoxin reductase 1-3, and several others of lesser significance [137]. In addition, the iodothyronine deiodinases active in thyroid hormone synthesis are selenoproteins [137].

1.3.2 Cellular pharmacology of selenite – uptake into the cell

In experiments *in vitro*, the metabolism of selenium compounds takes place mainly in the intracellular compartment, due to the requirement for participation of specialised enzymes, as discussed above. Selenite is a possible exception; this molecule is unstable and might react with exposed thiols on extracellular proteins. In a comparison between the toxicity of selenite when pre-incubated in serum at 37°C for 24 h or added directly to the cell culture medium, no difference could be seen (Nilsonne, unpublished).

The kinetics of uptake and retention is therefore the first consideration in an explanatory model for selenium cytotoxicity. This has been the focus of early studies using radiolabeled selenite (^{75}Se) in cell cultures. Thus, Morrison reported in 1988 [138] that the retention of selenite correlated to growth inhibition in 13 tested cell lines with r^2=0.6. In the same year, this group showed that detached cells dying from selenite exposure contained concentrations on the order of tenfold higher than adherent cells [139]. The retention was found to correlate to cell cycle phase; cell populations in a logarithmic growth phase demonstrated an even distribution of selenite between cells, whereas confluent cultures showed great variability and a higher overall retention [140]. In a limited time-course experiment, it was found that maximum retention was achieved already after 2 h [141].

MacVicar and Frenkel confirmed these results in 1993, reporting also that cells with a greater plating density were more sensitive to selenite [142]. Interestingly, this study found no correlation between selenite sensitivity and intracellular glutathione levels, but a strong correlation to selenite uptake.

In an investigation of the kinetics of selenite uptake in erythroleukemia cell lines using inductively coupled mass spectrometry, two patterns were seen depending on the dose of selenite [143]. Cells treated with 5 μM selenite reached a plateau after approx. 8 h, while those treated with 50 μM continued to take up selenite for 96 h and reached 100-fold higher intracellular concentrations. These results suggest the existence of a specific mechanism to regulate selenite uptake, while high concentrations may cause unspecific influx across the plasma membrane.

The existence of a specific transporter protein is also supported by data from non-human cell systems. The unicellular alga *Chlamydomonas rheinhardtii* has a fast transport mechanism operating in nanomolar concentrations and a slow transport mechanism operating in micromolar concentrations, also suggesting the presence of a specific transporter [144]. One investigation from 1985 showed that in *E. coli*, selenite is taken up by the same mechanism that imports sulphate and selenate [145]. The affinity for sulphate was 37 times that for selenite. In a study of *Selenomonas ruminantia*, on the other hand, selenite uptake could be inhibited by sulphite but not by sulphate [146], and in a study of *Saccharomyces cerevisiae*, two distinct kinetic phases were also found depending on the concentration of selenite [147].

In an elegant study published recently [148], Olm and co-workers have shown that cells secreting thiols, mainly in the form of cysteine, have a greater uptake of and sensitivity to selenite. This mechanism is due to overexpression of MRP family proteins, mediating cysteine export, and of the x_c^- cystin/glutamate antiporter for uptake of cystin, which is then intracellularly reduced and exported. According to this model, selenite is reduced to selenide in the extracellular compartment and then taken up by a still unknown import mechanism.

This model has similarities to one proposed by Tarze et al [149] to explain the mechanism of selenite cytotoxicity in *Saccharomyces cerevisiae*. The model is also supported by the findings of Ganyc and Self [150], who compared the uptake of selenite and selenide and found that selenide (or, at the very least, a reduced form of selenite)

was actively taken up by keratinocytes, and that the uptake could be inhibited by molybdate, chromate, and ATPase inhibition. The findings are also consistent with the reports by Batist et al. that the cytotoxicity of selenite is increased when glutathione is added to the culture medium [151], and by Caffrey and Frenkel [152] that depletion of glutathione in the medium decreases selenite toxicity, but that the toxicity can be restored by preincubation of selenite with glutathione.

On a macroscopic level, selenite has a strong affinity for tumour tissues, as is evidenced by scintigraphic studies performed using ^{75}Se radioselenite between 1960 and 1980. In a number of independent investigations, selenite gave a strong signal for tumours of any histogenesis, including malignant mesotheliomas, indicating that there is a mechanism for selenium uptake and retention that is common to most neoplastic processes [153-160]. The difference in signal strength between tumour and normal tissue, measured using the technology of that time, allows the estimation that the concentration of selenium was about 20- fold greater in the tumours (Martin Ingvar, personal communication). Kuikka and Nordman investigated tumour specimens from patients that had recently undergone ^{75}Se-scintigraphy *ex vivo*, and found a 5-8 times greater selenium content in the tumour tissue than in biopsies from the subcutaneous fat [161].

High concentrations were also seen in the liver and in inflammatory processes, e.g. abscesses, post-radiation fibrosis, and infarctions [155-157]. In light of the findings by Olm et al, discussed above, it is tempting to suggest a common mechanism for selenium enrichment, in that both tumours and many focal infections and inflammatory processes may be hypoxic, leading to reducing extracellular conditions. Curiously, the only quantitative investigations of the redox potential in tumours were carried out in the 1950's, These investigations did showed a markedly lower redox potential in tumours compared to other tissues, meaning that the tumour microenvironment was reducing [162, 163].

The main reasons for discontinuation of research into ^{75}Se selenite as a radiographic isotope were the insufficient specificity of the diagnostic method and the comparatively long half-time of selenite in the body (dual kinetics with a rapid excretion of $t_{1/2}$ around three days and a slow phase with $t_{1/2}$ around 150 days, presumably due to unspecific incorporation during protein synthesis), leading to a high radiation dose [164, 165]. Possibly, selenite will reemerge as a radiographic compound, as it is now possible to produce ^{73}Se-selenite and detect it using positron emission tomography, yielding higher sensitivity with far smaller doses than the older scintigraphic techniques [166, 167].

It should be added that selenite, when injected into the bloodstream, will be rapidly reduced to selenide in erythrocytes and in the liver [168]. Much of the added dose will then bind to thiols on various proteins, including glutathione, haemoglobin, and albumin [169, 170]. How much of an intravenous dose of selenite will reach a tumour before being metabolised in this manner remains an open question.

1.3.3 Cellular pharmacology of selenite – intracellular activity

In an attempt to define the fundamental principle of pharmacology, Paul Ehrlich in 1913 coined the phrase "Corpora non agunt nisi fixata"[2], meaning that a drug only exerts its effect when bound to its receptor [171]. Ehrlich himself was quick to point out that there are exceptions to this rule; these exceptions have however turned out to be very rare. Selenite and its chief metabolite, selenide, appear to be such exceptions, lacking a specific receptor.

The cytotoxic effect of selenite is mediated by oxidative stress. Many independent researchers have confirmed this hypothesis numerous times by the intervention with different antioxidants including ascorbic acid, catalase, superoxide dismutases, superoxide dismutase mimetics, superoxide scavengers, glutathione, glutathione peroxidase, deferoxamine, and N-acetylcysteine [172-182].

As mentioned above, selenite is metabolised to selenide. Unlike the conversion of selenoamino acids, the rate of this reaction is limited only by the local availability of electron donors. It is therefore possible for large amounts of selenite to reduce into selenide in a short time, while oxidising free thiols in the cytoplasm.

Selenide is a reactive molecule, reacting rapidly with free thiols, especially in glutathione, to form disulfides and selenium trisulfides [133]. These are again reduced, leading to a cycling where free thiols may be rapidly consumed [183]. The resulting oxidation leads to the formation of disulphide bridges interfering with the proper conformation of intracellular proteins, leading to inhibition or inactivation of their enzymatic function. This effect has been shown e.g. in DNA topoisomerases [184], caspases, and JNK [185, 186].

Selenodiglutathione may also be converted into colloidal selenium [187], which can be present in sufficiently high amounts in cells after selenite treatment to give the cell pellet a characteristic brick-red colour (Nilsonne, unpublished).

1.3.4 Induction of apoptosis by selenite

Much research on the cytotoxicity of selenite has revolved around its ability to induce apoptosis, and the signaling mechanisms involved in this process. There are three implicit reasons for this preoccupation:

- The ability of selenite to induce apoptosis suggests a specific mechanism rather than a general toxic effect
- In cancer treatment, apoptotic cell death in the tumour is far less of a burden for the patients compared to necrotic cell death, as the apoptotic cells can be phagocytosed locally instead of spilling their contents and causing local inflammation and a great burden of clearance for the liver and kidneys.

[2] This is a play on the older saying "corpora non agunt nisi liquida", which refers to the well-known phenomenon that many chemical reactions will only take place between compounds dissolved in the same fluid.

- Most currently used chemotherapeutic drugs induce DNA damage and lead to apoptosis.

Apoptosis continues to be a poorly defined process [188, 189]. Many hallmarks of apoptosis are well known, e.g. mitochondrial depolarization, caspase activation, and nuclear fragmentation [190]. However, it is clear that not all of these classical hallmarks need to be present at any stage of apoptosis. Different types of programmed cell death independent of caspase and of other mediators have been demonstrated [191].

Many independent reports have shown that selenite increases the expression of apoptotic markers in a great variety of cell types [174-176, 178, 179, 181, 182, 192-202]. However, cell death without apoptotic marker expression was also often observed. It is therefore likely that apoptosis induces cell death both by apoptosis and by necrosis, and that individual cells may show activation of the apopototic signalling and execution pathways to a varying extent after selenite treatment.

The exact nature of selenite-induced apoptosis signalling appears to differ between cell types. The main apoptosis mediators have by now been relatively well studied in different model systems. With regard to p53, the "master regulator" of cell death, several studies have shown induction after selenite treatment [179, 182, 194, 199, 201, 203, 204]. In one study, siRNA against p53 decreased the cytotoxic effect of selenite [199]. Another report showed that selenite caused p53 activation specifically by inducing phosphorylation of serines 20, 37, and 46 [205]. However, p53-independent selenite cytotoxicity has also been reported [203].

The mitogen-associated protein kinase (MAP kinase) pathways encompass six different signalling pathways, mediated by functionally similar serine/threonine protein kinases and involved in proliferation, differentiation and cell stress signalling [206-208]. Two of these pathways are particularly important for stress signalling and apoptosis regulation: the JNK (c-Jun N-terminal Kinase) and p38 pathways. There is considerable crosstalk between these pathways, and it is possible that a network is a better model for visualising the signalling interactions than linear pathways (see section 2.3.2).

Following selenite treatment, MAP kinase activation has been variable in different cell systems. Activation of p38 has been seen quite consistently [179, 201, 203], although these studies are mainly correlative and do not attribute causality to p38 activation in signalling. When it comes to JNK, earlier studies diverge, finding both activation [201], and no activation [179].

Caspases, the canonical apoptosis effector proteins, have also been found to be activated in response to selenite in some cell systems [174, 182, 199, 202] but not in others [179, 180, 192]. As mentioned above (section 1.3.3), selenite can inhibit caspases by thiol oxidation. This effect could explain the lack of caspase activation in some systems, and is consistent with the pattern of partial apoptosis induction often seen after selenite treatment.

In addition to these findings concerning apoptosis signalling, there have been reports that selenite induces autophagy, and that inhibition of autophagic signalling pathways increase the apoptotic fraction [209].

1.3.5 Determinants of selenite cytotoxicity

Since selenite does not have a specific molecular target, efforts to find predictors of the response in different cell populations have largely been frustrated. As mentioned above, the cellular uptake and retention of selenite was implicated as a determinant at an early stage [138], and this position has recently been vindicated [148].

Much interest has focused on the cellular redox enzyme systems as determinants of selenite cytotoxicity. The two main redox systems are the glutathione system, comprising glutathione, glutathione peroxidase, and NADPH, and the thioredoxin system, comprising thioredoxin, thioredoxin reductase, and NADPH. Of the two, the glutathione system is quantitatively highly dominant and has broader substrate specificity.

Thioredoxin reductase is upregulated in malignant mesothelioma, as we and others have shown using immunohistochemistry (**paper I**, [210]). Microarray studies of gene expression in mesothelioma have arrived at the same result [101, 211, 212]. At one point, we hypothesised that the high levels of thioredoxin reductase would sensitise the cells to selenite, by facilitating the redox cycling which generates oxidative stress [213]. On the one hand, the thioredoxin system protects the cells against the thiol oxidation that results from selenite-induced oxidative stress. On the other hand, by reducing the thiols it regenerates the substrate for redox cycling by selenite [214]. Hence, we used the simile of a double-edged sword in **paper I**. Later data, however, suggest that the protective effect of thioredoxin system activity is dominant over any deleterious effects – the edges of the sword are far from equally sharp. Overexpression of TrxR had a protective effect in one study [215]. Inhibition of thioredoxin reductase by Auranofin enhanced cell death in another study [216]. This is consistent with our observation in **paper I** that cytotoxicity was greater in the cell line with lower TrxR activity.

Results of similar studies on the glutathione system have been contradictory. Correlative studies matching glutathione levels to selenite cytotoxicity have found no relationship [178]. Glutathione depletion has been shown to lead to both increased [217, 218] and decreased [197] sensitivity to selenite. In one study, both up- and downmodulation of glutathione caused increased cytotoxicity of selenite [219].

We have also investigated whether the proliferation rate or plating density affects sensitivity (**paper III**), but we found no such correlation.

Thus, it seems that cellular uptake and retention of selenium after selenite treatment, mediated by extracellular thiols, is the best determinant currently known for selenite toxicity.

1.4 NATURAL KILLER CELLS IN ANTICANCER THERAPY

Natural killer (NK) cells are a special class of lymphocytes, belonging neither to the B nor the T subset. The name "natural killer cell" was coined by Eva Klein [220], and refers to the capacity of NK cells to kill target cells without prior activation by cytokines, if the target cell does not express inhibitory ligands, primarily major histocompatibility complex (MHC) class 1 proteins. Downregulation of MHC class 1 causes cells to avoid displaying immunogenic proteins in cases of virus infection or neoplastic transformation. NK cells are therefore particularly important in these contexts, where so-called "missing-self recognition" enables accurate recognition and killing of deleterious host cells.

The activation of NK cells is complex and depends on a balance of activating and inhibiting factors related both to the target cell and to the extracellular milieu. Important activating receptors include DNAX-accessory molecule-1 (DNAM-1), NKG2D, some of the killer Ig-like receptors (KIRs), natural cytotoxicity receptors (NCR), and CD94/NKG2C, and the concurrent activation of at least two receptors is required for NK cell activation [220]. The main inhibitory ligands are HLA class I family members, including HLA-E, recognised by receptors including leukocyte Ig-like receptor (LIR), some of the KIRs, and CD94/NKG2A [221]. In addition, NK cells are activated by Fc immunoglobulin fragments on opsonised cells, as well as by proinflammatory cytokines such as interleukin-12 and -18 [222].

The cytotoxic effects of NK cells are mediated by perforin and granzyme, two proteins stored in cytoplasmic granules of the NK cell [222]. Perforin acts by inserting itself into the plasma membrane of the target cell and forming a pore [222]. Granzyme, on the other hand, is a protease, which exerts its effects after entering through the pore by cleaving procaspases in the target cell cytoplasm, thereby activating apoptosis signalling [111, 112].

2 PRESENT INVESTIGATION

2.1 AIM OF THE STUDY

Improved therapeutic options for malignant mesothelioma are urgently needed. New drugs with greater efficacy would be highly valuable. It is, however, important to note that several existing drug combinations yield response rates of 30-40%, possibly with a considerable lack of overlap [223]. Therefore, if it were possible to determine which treatment regime would be most useful for each individual patient, better results could be expected. In addition, some patients might be spared the effort of going through arduous treatment cycles when the added benefit is very small.

The overall aim of this project was to investigate the anticancer effect, mechanisms, and possible utility of sodium selenite as a new drug against malignant mesothelioma, as well as the feasibility of using the differentiation state of cells to predict treatment response. The thesis work has been divided into four sub-projects, the aims of which were as follows:

Paper I: To investigate the ability of sodium selenite to induce cell death and apoptosis in mesothelioma cells; the tumour- and phenotype-specificity of selenite toxicity; the effects of a combination of selenite and a conventional cytotoxic drug; and whether selenite toxicity is mediated, in this experimental system, by oxidative stress.

Paper II: To investigate the apoptosis signalling in mesothelioma cells of epithelioid and sarcomatoid phenotypes, with respect to the regulation of a number of key signaling molecules including p53, JNK, p38, Bax, Bcl-XL, caspases and cathepsins.

Paper III: To investigate whether selenite has synergistic effects with other cytostatic drugs in mesothelioma cells, and whether the differentiation state of the cells is a predictor for the sensitivity to selenite and other drugs.

Paper IV: To investigate the effects of selenite on NK-cell mediated recognition and killing of mesothelioma cells, with a particular focus on the down-regulation of HLA-E in response to selenite treatment.

2.2 REMARKS ON METHODOLOGY

2.2.1 Cytotoxicity assays

Cytotoxicity has been assayed mainly by the WST-1 assay, which measures the metabolic activity of cell populations. It is a viability test rather than a cytotoxicity test, and can therefore determine the viability of a population relative to control. It can, however, not distinguish between a cytotoxic effect where cells have been killed, and a cytostatic effect, where proliferation has been impaired.

It could be hypothesized that the result of the WST-1 assay is confounded by oxidative stress, which is induced by selenite and several of the other compounds that have been tested in the course of this work. This hypothesis has been investigated in a recent study

[224] which compared several viability tests after treatment with different selenium compounds. The WST-1 assay consistently outperformed other assays in that it yielded higher viability estimates for untreated cells and a more dynamic response to increasing concentrations of toxic drugs. In some cases, however, the WST-1 assay yielded a paradoxical increase with increasing drug concentrations, which is attributed by the authors to an increase in the metabolic activity. We have observed such increases when cells have been treated with low doses of selenite, and we have suggested in **paper I** that this effect is due to increased viability because low doses of selenium may be growth stimulatory. In the light of these more recent observations, an alternative interpretation is that the toxic effects of selenite manifested themselves as an increased metabolic activity at low doses.

2.2.2 Apoptosis assays

Many different methods to determine apoptosis have been used in this work. For quantitative comparisons, we have relied mainly on flow cytometric methods using Annexin-V in conjunction with a DNA staining marker such as propidium iodide or 7-aminoactinomycin D [189, 190]. This approach is valuable because it offers a way to validate findings from cytotoxicity assays, while giving a readout for both apoptotic and non-apoptotic cell death.

We have also (in **paper I**) used a purely morphological technique where apopototic cells were defined as those with pycnotic (i.e. shrunken and hyperchromatic) nuclei. This is the oldest available method for apoptosis detection, but it is nevertheless still considered one of the most robust methods [225]. An important limitation is the subjectivity of morphological measurements, leading to interobserver variation. The evaluation of slides in this experiment was performed by a single observer. Variation due to subjectivity was instead mitigated by the joint assessment of several slides, chosen at random, by three observers, in order to establish a common norm for evaluation. All slides were blinded.

The main weakness of these analyses is that they offer only a snapshot of living and dead cells in the population. We have shown by means of mathematical simulation that changes in the kinetics of apoptosis may lead to large over-or underestimates unless kinetics are explicitly taken into consideration (Nilsonne et al, unpublished). Therefore, these quantitative comparisons must be made with caution.

An alternative to single-cell based methods such as the above-mentioned techniques is to assay a cumulatively increasing product of apoptosis from the cell medium. In this work (**paper II**), we have used the M-30 Apoptosense ELISA. This assay measures specific fragments of cytokeratin 18 resulting from cleavage by caspases, particularly caspases 3 and 9. Consequently, the assay is dependent on both the expression of cytokeratin 18, and on the extent to which apoptosis is mediated by these caspases. Caspase-independent apoptosis will lead to negative results. In order to correct for differences in baseline cytokeratin expression we have expressed the results as *fold change* in treated cells compared to untreated cells, instead of directly comparing the results from different cell lines.

2.2.3 Measurement of oxidative stress

Several methods exist for the determination of oxidative stress, all with their respective advantages and limitations. In this work, two methods have been used: dichlorofluorescein (DCF) staining and measurement of free thiols by the DTNB assay.

DCF staining was evaluated by confocal microscopy, which is a sensitive technique capable of detecting very weak fluorescence signals. However, this high sensitivity requires a high stringency with respect to negative controls: all samples and controls must be prepared at the same time, under identical conditions, and the exact same instrument settings with respect to laser intensity and amplitude gain must be applied throughout. This may lead to problems such as signal saturation. A further challenge is the apparent time-dependence of DCF fluorescence after selenite treatment. In this work, we have made conservative interpretations of the DCF experiments by drawing only qualitative conclusions, noting that staining intensity was greater or smaller when comparing two samples, but not by how much.

While DCF fluorescence is primarily a measure of hydrogen peroxide (H_2O_2) generation from the superoxide anion (O_2^-) [226], thiol oxidation can be considered an independent outcome parameter, related but by no means identical to superoxide generation. Measurement is comparatively simple and rests on the use of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)), which forms the chromophore 5-mercapto-2-nitrobenzoic acid upon reaction with sulfhydryl groups. Readout is performed at 412 nm with a spectrophotometer and the concentration is calculated based on the extinction coefficient ε ~13,600 $cm^{-1}M^{-1}$ [227]. This readout is then related to the protein concentration, as determined by the BioRad assay. DTNB conjugates of glutathione and other thiols can also be separated by high-pressure liquid chromatography and quantified based on their absorbance for greater sensitivity and accuracy.

In addition to the abovementioned assays, we have used ascorbic acid as an antioxidant in conjunction with viability and apoptosis tests, establishing a causal role for oxidative stress in processes leading to cell death.

2.2.4 Immunohisto- and cytochemistry

These antibody-based and principally similar methods have been used both for target validation in patient material and for evaluation of experimental endpoints in cells from tissue culture. In both methods, target detection depends on antibody binding. Here, it is necessary to ensure specificity, by means of a negative control, and sensitivity, by means of a positive control. The specificity of the antibody, unless it has been previously tested, can be further verified by techniques such as co-immunoprecipitation with the antigen (if the antigen is available), and staining attenuation by preincubation of the antibody with external free antigen before staining.
Interpretation of staining results is performed by a microscopist whose subjectivity is a possible source of bias. Therefore, all staining results were evaluated by at least two, and usually three, independent observers, and with blinding of the slides where possible. It was not possible to blind slides with respect to different diagnoses when these were

revealed by the morphology of the stained cells. When the results were qualitative in nature (such as whether a certain tissue stained positively or negatively), divergent opinions were settled by joint evaluation and discussion between the observers. When the results were quantitative in nature (such as number of positive/negative cells), the results from the different observers were added and summary statistics (positive fraction, confidence interval etc) were based on this aggregated dataset. All observers counted cells in a predefined number of microscopic fields of view, leading to nearly balanced cell counts. However, the slight differences in frequencies of counted cells precluded the calculation of formal interrater agreement measures such as the κ statistic.

2.2.5 Flow cytometry

Flow cytometry was most often performed using antibodies with fluorochromes for detection, although some assays were based on low-molecular fluorochromes with different staining principles (i.e. probes for mitochondrial membrane potential). With antibody staining, all the considerations discussed for immunohisto- and cytochemistry apply.

3 RESULTS AND DISCUSSION

3.1 Selenite in mesothelioma therapy

We have shown that selenite decreases the viability of mesothelioma cells (**paper I**). The mode of cell death showed many characteristics of apoptosis, although not in all cells (**papers I** and **II**). Mesothelioma cells were shown to be more sensitive than benign mesothelial cells in culture (**paper I**). This observation establishes that the basic criterion for a candidate anticancer drug is fulfilled; namely, that it has a selective effect on the tumour cells. Depending on the mode of administration, dose-limiting side-effects may of course arise due to even higher sensitivity in other benign cell types. Malignant mesothelioma is one of the few solid tumours where topical chemotherapy is practical, in the form of an intracavitary infusion into the pleura or peritoneum [228, 229]. This approach might result in a far higher local availability of selenite, as it would not undergo metabolism in the liver and erythrocytes before reaching the tumour (as discussed in section 1.3).

In **paper I**, we reasoned that thioredoxin reductase (TrxR) could be a drug target for selenite, and we showed that the activities of both TrxR and glutaredoxin reductase decreased after selenite treatment. As has been discussed in section 1.3, this hypothesis is, in light of subsequent findings in other systems, unlikely to hold. However, TrxR remains a strong candidate drug target for mesothelioma and other cancers [230]. The observed inhibition of TrxR activity could possibly be explained by relatively non-specific effects of oxidation, leading to the formation of intra- and intermolecular disulfides or mixed disulfides/selenides, disrupting the tertiary structure of the TrxR protein [231, 232].

In **paper III**, we investigated the effect of selenite compared to other cytostatic drugs, and found a greater decrease in viability at theoretically relevant dose ranges. We also investigated synergistic effects between selenite and other anticancer drugs. From *a priori* theoretical considerations, a synergistic effect between selenite and Pemetrexed might be expected, since Pemetrexed decreases the availability of methyl groups required for the detoxification of selenide into methylselenol. However, no such effect was observed.

In **paper IV**, we have shown that selenite alters the immune phenotype of mesothelioma cells, leading to increased recognition by NK-cells and subsequent degranulation and killing of the tumour cell by the NK cell. The NK cell inhibitory ligand HLA-E is downregulated by selenite, and this regulation takes place on the level of protein synthesis, as evidenced by RT-PCR showing that mRNA expression is constant, while intracellular and surface protein levels decreased synchronously. HLA-E is a labile molecule, with a half-time of only 4-6 h. Therefore, we hypothesise that it functions as a rapidly onsetting marker for cell stress. These results indicate that selenite treatment *in vivo* will have the additional benefit of immune mechanisms on top of the direct cytotoxic effects evidenced in **papers I-III**.

The doses of selenite used throughout these investigations are within the tolerable range in humans [101, 233-235]. Therefore, it is reasonable to extrapolate the effects of selenite on mesothelioma cells to *in vivo* conditions, while keeping in mind that pharmacokinetics may still cause profound differences in effect.

The results of these *in vitro* investigations establish a basis for further studies of the safety and efficacy of selenite against malignant mesothelioma in a clinical setting. The establishment of a viable translational strategy for the testing of selenite against malignant mesothelioma is a weighty responsibility for those who will continue in this line of research.

3.2 Individualized therapy for mesothelioma

In **paper I**, we hypothesised that the differentiation state of mesothelioma cells (epithelioid vs. sarcomatoid) predicts response to selenite treatment. This reasoning was based on the following arguments:

- Previous studies of gene expression have found robust biological differences between the two phenotypes, suggesting that they have different tumour-driving mechanisms [236].
- In **paper I**, experiments on a model system for mesothelioma differentiation comprising one epithelioid and one sarcomatoid cell sub-line showed that the sarcomatoid cell sub-line was more sensitive to selenite. A previous study showed that the reverse was true for proteasome inhibitors [237].

In **paper III**, investigated the association of differentiation state to drug sensitivity, by comparing six cell lines, of which three displayed epithelioid differentiation and three were of sarcomatoid phenotype. Analyses of variance (ANOVA) showed an extremely weak correlation between differentiation state and drug sensitivity, thus disproving our hypothesis that differentiation predicts sensitivity to the tested drugs.

New predictors for clinical use must evidently be sought elsewhere. A promising approach might be to study the functional characteristics of primary tumour cells derived from the tumour e.g. from a pleural effusion [189]. Such efforts are ongoing in our own laboratory and elsewhere.

3.3 Apoptosis regulation: How the cell makes a decision

Programmed cell death encompasses a spectrum of interrelated processes, whose definitions are not completely clear [189]. Traditionally, *apoptosis* denotes programmed cell death characterized morphologically by nuclear shrinkage, pycnosis, and fragmentation, and characterized biochemically by DNA strand breaks and activation of a particular set of proteases (most often the caspases, although other proteases may perform the same function). Many other well-studied features may be present, such as depolarization of the mitochondria, and externalisation of phosphatidyl serine on the plasma membrane. As no distinct definition of apoptosis exists [238], many investigators have stressed the importance of assaying several (≥ 3) signs of apoptosis.

Apoptosis and *programmed cell death* are not fully synonymous concepts. *Autophagy* is another form of programmed cell death, which is characterised by cellular autocatalysis by means of lysosomal degradation. During autophagy, the lysosomes expand in size and are termed "autophagosomes". A third form of programmed cell death, which was recently proposed, is *entosis*, where cells actively contribute to becoming phagocytosed and degraded by the engulfing cell [239]. This phenomenon is well known from clinical cytological observations, where so-called "engulfment" phenomena can be seen among tumour cells [188].

In addition, cells may die from the inability to maintain their normal homeostasis. This can take the form of either *necrosis* or *mitotic catastrophe* [240, 241]. In some cases, a cell may begin to execute a cell death programme, but become unable to complete it, and the cell death in this case will lie somewhere in-between necrosis and programmed cell death. After treatment with selenite, cell populations show a distribution of cell death with variable activation of apoptosis signalling pathways. We hypothesise that in these cases, many cells enter necrosis before being able to finish the apoptotic process even though it had already started.

From the point of view of network organisation, apoptosis regulation is very interesting. Several broad classes of apoptosis stimulating signals are recognised, including genotoxic stress of many kinds, extrinsic activation through the Fas pathway, and hormonal/morphogenic cues during development. The relationship between these cues and apoptosis is non-linear. To ensure proper function, the signalling network has to disregard small stimuli, but once a certain threshold is exceeded, the activation must be complete. In other words, the network must be *bistable* and allow only an "on" and an "off" condition [242]. Manintenance of the "on" condition is probably achieved through powerful positive feedback signals on several levels. One well-known positive feedback mechanism is the activation of caspases by other caspases through the cleavage of inactive procaspases. Other positive feedback mechanims are likely to operate on mediators upstream of the caspases [242].

Due to the topology of the network, it is difficult to say, for many mediators, whether they are pro- or antiapoptotic. Both JNK and p38 have been pointed out as pro-apoptotic by a considerable literature, while an equally considerable literature has claimed that they are anti-apoptotic [30]. This illustrates the difficulties in interpretation that arise from assuming linear relationships in a complex network.

In **paper II**, we have showed that inhibition of a number of apoptosis signalling mediators had very little effect on apoptosis induction by selenite. The network was apparently robust to our perturbations. Two explanations are possible: either a mediator in question was not active in apoptosis signalling after selenite induction, or it was functionally redundant. Of the investigated mediators, we can be fairly certain that p53 was not active in signalling, as we also demonstrated that selenite caused p53 to lose its activity as a transcription factor. The cases of JNK and p38 as well as the cathepsins are not as straightforward. Here, our experimental controls show that p38 and the cathepsins, but not JNK, were active in signalling for the apoptosis that occurred spontaneously without selenite treatment. It is possible that these cells, which carry many genetic alterations , do not use JNK signalling at all. With respect to p38 and the

cathepsins, it is more likely that these mediators are either redundant or inhibited by selenite.

We conclude, therefore, that the apoptosis signalling network of the mesothelioma cells under study was highly robust to perturbation of the several key mediators tested, and that contributing factors to this robustness may include prior inactivation of apoptosis pathways acquired during the course of tumourigenesis, inhibition of pathways by the action of selenite, and a network architecture with a high degree of functional redundancy.

4 ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to:

My supervisor **Katalin Dobra** for offering me the freedom to pursue my own path, even when it was long and tortuous. My co-supervisor **Anders Hjerpe**, for teaching me how to do science, and how to have fun while doing it.

My fellow group members, past and present. **Fang Zong**, for sharing the many joys and burdens of the time as a PhD student. **Adam Szulkin**, for all the things we have learned together during close collaboration over the years. **Filip Mundt**, for all the good discussions and all the good work together. **Mervi Nurminen**, for keeping your eye on us and your wing over the entire lab. **Nina Wolmer Solberg**, for showing me the ropes when I was clueless. **Eva Dárai-Ramqvist**, for bringing new techniques and approaches into the group. **Pega Souri,** for injecting your glamour and sunshine. **Anna Zoltowska**, for good work on your summer project. **Xiaojuan Sun**, and all other past members of the group, for pleasant company and valuable teamwork.

Our collaborating partners. **Mattias Carlsten**, for showing me what excellent research really is about. **Niklas Björkström**, for many great science discussions and chess games. **Monika Simonsson**, **Kalle Malmberg** and **Hans-Gustaf Ljunggren**, for fruitful collaboration. **Mikael Björnstedt**, for introducing me to the field of selenium research. **Anna-Klara Rundlöf** and **Aristi Fernandes**, for teaching me new methods and always extending a helping hand. **Eric Olm**, for good cooperation and for adding a bit of spirit to the department. **Markus Selenius**, for good teamwork. **Christina Nyström**, and other past and present members of the Björnstedt lab, for contributing to a pleasant and generous atmosphere in the department of Pathology. **Måns Magnusson**, for friendship and valuable advice on statistics. **Kjell Hultenby**, **Klaus Leifer**, and **Ernesto Coronel**, for enthusiastic and expert cooperation regarding electron microscopy. **Mats Lekander** and **John Axelsson**, for welcoming me into a tremendously exciting project that may become an auspicious start for further work. All other co-authors, for our mutual efforts.

My academic leaders. University President **Harriet Wallberg-Henriksson**, for many interesting discussions about where the Karolinska Institutet is heading, and why. Department head **Lennart Eriksson**, for generously sharing your ideas, both in the field of cancer biology, and in the field of hospital and laboratory management and organization. Division head **Göran Andersson**, for your contributions towards making the department a stimulating place to work.

My seniors, colleagues, and friends working in the division of pathology. **Kristin Gustafsson**, for walking side by side with me as a PhD student and sharing each others' triumphs and setbacks. **Birgitta Sander** and **Birger Christensson**, for always taking an interest in my data when I needed input, and for setting an example of stringency and honesty in science. **Pernilla Lång** and **Maria Norgård**, for cooperating to make important things function in the department. **Barbro Ek-Rylander**, for believing that teaching is important, and generously inviting me to instruct your many students.

Gareth Morgan and **Björn Rozell**, for sharing your knowledge of and appreciation for art and science. **Åsa-Lena Dackland** and **Åsa Jeppson-Ahlberg**, for expert assistance with flow cytometry. **Carmen Flores-Staino**, **Gunilla Fahlström,** and **Ingrid Norman**, for expert assistance with immunohisto- and cytochemistry. All other past and present members of the division of pathology.

The physicians of the laboratory for clinical pathology and cytology, who have generously shared their knowledge and expertise with me. In addition to the several already mentioned above in other capacities, I would like to thank **Margareta Blåsjö, Jan Ericsson, Anders Sundberg, Håkan Hanson, Sam Ghazi, Bela Bozoky, Nikos Papadogiannakis, Inmaculada Ribera-Cortada, Anna Tolf, Barbro Lundh-Rozell, Inger Nennesmo, Britta Krynitz, Attila Szakos, Nelly Penagos-Falk, Henrik Ingelman-Sundberg, Annika Wernerson, Servet Eken, Andrea Ekroth,** and **Magnus Söderberg.**

Mentors and guides in science and medicine. **Martin Ingvar**, for showing me the meaning of intellectual courage. **Sven Grützmeier**, for many insights about becoming and being a doctor, from the point of view of practical experience. **Johan Christensen**, for sharing his experiences as a physician in finance and industry. **Hans Aili**, for interesting discussions about how the modern university came into being, and for accidentally causing me to understand scientific positivism. **Christer Ekdahl**, for being my guide through the great adventure of discovering the fundamental laws of nature, as my science teacher in school. **Ingemar Ernberg**, for single-handedly infusing the Karolinska Institutet with a discussion of modern biology in which I could participate as a graduate student, and where I have for the first time encountered many of the most transformative ideas of the last decade.

Friends and fellow travelers on the road to Great Medicine: **Katarina Grossmann**, **Andreas Hurme-Lundin**, **Stina Bjuggren Zelano**, **Johan Zelano**, **Karin Sundström**, **Carl Savage**, **Louise Scheen**, **Yosef Tyson**, **Adina Welander**, **Mia Öhman**, **Ernesto Forsberg**, **Hampus Janko**, **Daniel Lindbom**, **Jeanna Joneberg**, **Magnus Frödin**, **Jens Andersson**, **Raffaella Crinelli**, **Clara Brandkvist**, **Johan Ejerhed**, **Elias Engström, Malin Georgsson**, **Carl Tosterud**, **Maija Andersson**, **Mårten Trotzig**, **Sandra Lockmer**, **Saga Nybäck**, **Anders Näsman**, **Viveca Gyberg**, and my other fellow students over the years.

My family and friends. My mother **Åsa** and my father **Ulf**. My brothers **Carl** and **Hjalmar**. **Lars Werdelin**. My grand-mother **Maud** and my uncle **Magnus**. **Patrik Ringström**, **Edward Klingspor**, **Eleonor Hjorth**, **Caroline Uggla**, **Ania Jonasson**, **Christian Antoni Møllerop**, **Antonia Wulff**, **Patrick Cedergren**, **Sara Folke**, **Olof Sundblad**, **Anders Davén**, **Eva Hesselmark**, **Oscar Lavelid**, **Göran Pallmar**, **Erik Lundin**, **Elin Lund Jungstedt**, **Evelina Lorentzon**, **Isak Ericsson**, **Christian Löf**, **Hanna Bäckmo**, **Lieven Kuppens**, **Per Nordlund, Louise Jörnhagen**, **Lisa Gemmel**, **Robin Zimmerman**, and everyone else who has supported me with their friendship and affection during the years that I have been occupied with this thesis.

5 REFERENCES

1. Mutsaers SE: **Mesothelial cells: their structure, function and role in serosal repair.** *Respirology* 2002, **7:**171-191.

2. Heldin P, Pertoft H: **Synthesis and assembly of the hyaluronan-containing coats around normal human mesothelial cells.** *Exp Cell Res* 1993, **208:**422-429.

3. Castor CW, Naylor B: **Characteristics of normal and malignant human mesothelial cells studied in vitro.** *Lab Invest* 1969, **20:**437-443.

4. Breborowicz A, Wisniewska J, Polubinska A, Wieczorowska-Tobis K, Martis L, Oreopoulos DG: **Role of peritoneal mesothelial cells and fibroblasts in the synthesis of hyaluronan during peritoneal dialysis.** *Perit Dial Int* 1998, **18:**382-386.

5. Casey RC, Skubitz AP: **CD44 and beta1 integrins mediate ovarian carcinoma cell migration toward extracellular matrix proteins.** *Clin Exp Metastasis* 2000, **18:**67-75.

6. Jones LM, Gardner MJ, Catterall JB, Turner GA: **Hyaluronic acid secreted by mesothelial cells: a natural barrier to ovarian cancer cell adhesion.** *Clin Exp Metastasis* 1995, **13:**373-380.

7. Kluge T, Hovig T: **The ultrastructure of human and rat pericardium. I. Parietal and visceral mesothelium.** *Acta Pathol Microbiol Scand* 1967, **71:**529-546.

8. Ishihara T, Ferrans VJ, Jones M, Boyce SW, Kawanami O, Roberts WC: **Histologic and ultrastructural features of normal human parietal pericardium.** *Am J Cardiol* 1980, **46:**744-753.

9. Cotran RS, Karnovsky MJ: **Ultrastructural studies on the permeability of the mesothelium to horseradish peroxidase.** *J Cell Biol* 1968, **37:**123-137.

10. Fedorko ME, Hirsch JG, Fried B: **Studies on transport of macromolecules and small particles across mesothelial cells of the mouse omentum. II. Kinetic features and metabolic requirements.** *Exp Cell Res* 1971, **69:**313-323.

11. Fukata H: **Electron Microscopic Study on Normal Rat Peritoneal Mesothelium and Its Changes in Absorption of Particulate Iron Dextran Complex.** *Acta Pathol Jpn* 1963, **13:**309-325.

12. Leak LV, Rahil K: **Permeability of the diaphragmatic mesothelium: the ultrastructural basis for "stomata".** *Am J Anat* 1978, **151:**557-593.

13. von Recklinghausen FV: **Zur Fettresorption.** *Arch Pathol Anat* 1863, **24:**172-208.

14. Nakatani T, Ohtani O, Tanaka S: **Lymphatic stomata in the murine diaphragmatic peritoneum: the timing of their appearance and a map of their distribution.** *Anat Rec* 1996, **244:**529-539.

15. Ohtani Y, Ohtani O, Nakatani T: **Microanatomy of the rat diaphragm with special reference to the lymphatics and mesothelial stomata.** *Ital J Anat Embryol* 1995, **100 Suppl 1:**143-153.

16. Shao XJ, Ohtani O, Saitoh M, Ohtani Y: **Development of diaphragmatic lymphatics: the process of their direct connection to the peritoneal cavity.** *Arch Histol Cytol* 1998, **61:**137-149.

17. Miura T, Shimada T, Tanaka K, Chujo M, Uchida Y: **Lymphatic drainage of carbon particles injected into the pleural cavity of the monkey, as studied by video-assisted thoracoscopy and electron microscopy.** *J Thorac Cardiovasc Surg* 2000, **120:**437-447.

18. Demetri GD, Zenzie BW, Rheinwald JG, Griffin JD: **Expression of colony-stimulating factor genes by normal human mesothelial cells and human malignant mesothelioma cells lines in vitro.** *Blood* 1989, **74:**940-946.

19. Lanfrancone L, Boraschi D, Ghiara P, Falini B, Grignani F, Peri G, Mantovani A, Pelicci PG: **Human peritoneal mesothelial cells produce many cytokines (granulocyte colony-stimulating factor [CSF], granulocyte-monocyte-CSF, macrophage-CSF, interleukin-1 [IL-1], and IL-6) and are activated and stimulated to grow by IL-1.** *Blood* 1992, **80:**2835-2842.

20. Offner FA, Obrist P, Stadlmann S, Feichtinger H, Klingler P, Herold M, Zwierzina H, Hittmair A, Mikuz G, Abendstein B, et al.: **IL-6 secretion by human peritoneal mesothelial and ovarian cancer cells.** *Cytokine* 1995, **7:**542-547.

21. Fujino S, Yokoyama A, Kohno N, Hiwada K: **Interleukin 6 is an autocrine growth factor for**

normal human pleural mesothelial cells. *Am J Respir Cell Mol Biol* 1996, **14:**508-515.

22. Betjes MG, Tuk CW, Struijk DG, Krediet RT, Arisz L, Hart M, Beelen RH: **Interleukin-8 production by human peritoneal mesothelial cells in response to tumor necrosis factor-alpha, interleukin-1, and medium conditioned by macrophages cocultured with Staphylococcus epidermidis.** *J Infect Dis* 1993, **168:**1202-1210.

23. Offner FA, Feichtinger H, Stadlmann S, Obrist P, Marth C, Klingler P, Grage B, Schmahl M, Knabbe C: **Transforming growth factor-beta synthesis by human peritoneal mesothelial cells. Induction by interleukin-1.** *Am J Pathol* 1996, **148:**1679-1688.

24. Versnel MA, Hagemeijer A, Bouts MJ, van der Kwast TH, Hoogsteden HC: **Expression of c-sis (PDGF B-chain) and PDGF A-chain genes in ten human malignant mesothelioma cell lines derived from primary and metastatic tumors.** *Oncogene* 1988, **2:**601-605.

25. Cronauer MV, Stadlmann S, Klocker H, Abendstein B, Eder IE, Rogatsch H, Zeimet AG, Marth C, Offner FA: **Basic fibroblast growth factor synthesis by human peritoneal mesothelial cells: induction by interleukin-1.** *Am J Pathol* 1999, **155:**1977-1984.

26. Gary Lee YC, Melkerneker D, Thompson PJ, Light RW, Lane KB: **Transforming growth factor beta induces vascular endothelial growth factor elaboration from pleural mesothelial cells in vivo and in vitro.** *Am J Respir Crit Care Med* 2002, **165:**88-94.

27. Valle MT, Degl'Innocenti ML, Bertelli R, Facchetti P, Perfumo F, Fenoglio D, Kunkl A, Gusmano R, Manca F: **Antigen-presenting function of human peritoneum mesothelial cells.** *Clin Exp Immunol* 1995, **101:**172-176.

28. Visser CE, Brouwer-Steenbergen JJ, Schadee-Eestermans IL, Meijer S, Krediet RT, Beelen RH: **Ingestion of Staphylococcus aureus, Staphylococcus epidermidis, and Escherichia coli by human peritoneal mesothelial cells.** *Infect Immun* 1996, **64:**3425-3428.

29. Visser CE, Steenbergen JJ, Betjes MG, Meijer S, Arisz L, Hoefsmit EC, Krediet RT, Beelen RH: **Interleukin-8 production by human mesothelial cells after direct stimulation with staphylococci.** *Infect Immun* 1995, **63:**4206-4209.

30. Klominek J, Robért KH, Hjerpe A, Wickström B, Gahrton G: **Serum-dependent Growth Patterns of Two, Newly Established Human Mesothelioma Cell Lines.** *Cancer res* 1989, **49:**6118-6122.

31. Maeda M, Johnson KR, Wheelock MJ: **Cadherin switching: essential for behavioral but not morphological changes during an epithelium-to-mesenchyme transition.** *J Cell Sci* 2005, **118:**873-887.

32. Hjerpe A, Dobra K: **Malignant mesothelioma--a connective tissue tumor with proteoglycan-dependent differentiation.** *Connect Tissue Res* 2008, **49:**249-251.

33. Clarke WC: **Experimental mesothelium.** *The anatomical record* 1915, **10:**301-316.

34. Lewis WH: **Mesenchyme and Mesothelium.** *Journal of Experimental Medicine* 1923, **38:**257-262.

35. Maximow A: **Über das Mesothel (Deckzellen der serösen Häute) und die Zellen der serösen Exsudate. Untersuchungen an entzündetem Gewebe und an Gewebskulturen.** *Arch Exp Zellforschung* 1927, **4:**1-42.

36. Stout AP, Murray MR: **Localized pleural mesothelioma: Investigation of its characteristics and histogenesis by the method of tissue culture.** *Arch Pathol* 1942, **34:**951-964.

37. Raftery AT: **Regeneration of parietal and visceral peritoneum: an electron microscopical study.** *J Anat* 1973, **115:**375-392.

38. Raftery AT: **Regeneration of parietal and visceral peritoneum. A light microscopical study.** *Br J Surg* 1973, **60:**293-299.

39. Bolen JW, Hammar SP, McNutt MA: **Reactive and neoplastic serosal tissue. A light-microscopic, ultrastructural, and immunocytochemical study.** *Am J Surg Pathol* 1986, **10:**34-47.

40. Foley-Comer AJ, Herrick SE, Al-Mishlab T, Prele CM, Laurent GJ, Mutsaers SE: **Evidence for incorporation of free-floating mesothelial cells as a mechanism of serosal healing.** *J Cell Sci* 2002, **115:**1383-1389.

41. Yanez-Mo M, Lara-Pezzi E, Selgas R, Ramirez-Huesca M, Dominguez-Jimenez C, Jimenez-Heffernan JA, Aguilera A, Sanchez-Tomero JA, Bajo MA, Alvarez V, Castro MA, del Peso G, Cirujeda A, Gamallo C, Sanchez-Madrid F,

Lopez-Cabrera M: **Peritoneal dialysis and epithelial-to-mesenchymal transition of mesothelial cells.** *N Engl J Med* 2003, **348:**403-413.

42. Connell ND, Rheinwald JG: **Regulation of the cytoskeleton in mesothelial cells: reversible loss of keratin and increase in vimentin during rapid growth in culture.** *Cell* 1983, **34:**245-253.

43. Mutsaers SE, McAnulty RJ, Laurent GJ, Versnel MA, Whitaker D, Papadimitriou JM: **Cytokine regulation of mesothelial cell proliferation in vitro and in vivo.** *Eur J Cell Biol* 1997, **72:**24-29.

44. Fotev Z, Whitaker D, Papadimitriou JM: **Role of macrophages in mesothelial healing.** *J Pathol* 1987, **151:**209-219.

45. Mutsaers SE, Whitaker D, Papadimitriou JM: **Mesothelial regeneration is not dependent on subserosal cells.** *J Pathol* 2000, **190:**86-92.

46. Männer J, Perez-Pomares JM, Macias D, Munoz-Chapuli R: **The origin, formation and developmental significance of the epicardium: a review.** *Cells Tissues Organs* 2001, **169:**89-103.

47. Mullen A: *An Anatomical Account of the Elephant Accidentally Burnt in Dublin on Fryday, June 17, in the Year 1681.* London: Printed for Sam Smith; 1682.

48. Bolk L: **Anatomische Bemerkungen über einen Fetus von Elephas Africanus.** *Versl Verg Wis-en Natuurk Afd* 1917, **25:**1228.

49. Short RV: **The peculiar lungs of the elephant.** *New Scientist* 1962, **316:**570-572.

50. West JB: **Snorkel breathing in the elephant explains the unique anatomy of its pleura.** *Respir Physiol* 2001, **126:**1-8.

51. Aristoteles: **De partibus animalium.** vol. 2.

52. Hillerdal G: **The Swedish experience with asbestos: history of use, diseases, legislation, and compensation.** *Int J Occup Environ Health* 2004, **10:**154-158.

53. Dioscorides. P: **De Materia Medica.** vol. 5. pp. 138:138.

54. Secundus CP: **Naturalis Historia.** vol. 19.

55. Ross M, Nolan RP: **History of asbestos discovery and use and asbestos-related disease in context with the occurrence of asbestos within ophiolite complexes.** In *Geological Society of America, Special Paper*, vol. 373; 2003.

56. Dogan M, Dogan AU: **Asbestos Mineralogy and Health Effects.** In *Malignant mesothelioma - Advances in Pathogenesis, Diagnosis, and Translational Therapies.* Edited by Pass H, Vogelzang NJ, Carbone M. New York: Springer; 2005: 209-224

57. Baris YI, Grandjean P: **Prospective study of mesothelioma mortality in Turkish villages with exposure to fibrous zeolite.** *J Natl Cancer Inst* 2006, **98:**414-417.

58. Baris YI, Sahin AA, Ozesmi M, Kerse I, Ozen E, Kolacan B, Altinors M, Goktepeli A: **An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Urgup in Anatolia.** *Thorax* 1978, **33:**181-192.

59. Wedler HW: **Über den lungenkrebs bei asbestose.** *Deutsches Archiv für klinische Medizin* 1943, **191:**189-209.

60. Wagner JC: **The discovery of the association between blue asbestos and mesotheliomas and the aftermath.** *Br J Ind Med* 1991, **48:**399-403.

61. Wagner JC, Sleggs CA, Marchand P: **Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province.** *Br J Ind Med* 1960, **17:**260-271.

62. Sébastien P, Bégin R, Case BW, McDonald JC: **Inhalation of chrysotile dust.** In *The Biological Effects of Chrysotile.* Edited by Wagner JC. Philadelphia: JB Lippincott; 1987: 19-29

63. Muscat JE, Wynder EL: **Cigarette smoking, asbestos exposure, and malignant mesothelioma.** *Cancer Res* 1991, **51:**2263-2267.

64. Carbone M, Pass HI, Rizzo P, Marinetti M, Di Muzio M, Mew DJ, Levine AS, Procopio A: **Simian virus 40-like DNA sequences in human pleural mesothelioma.** *Oncogene* 1994, **9:**1781-1790.

65. Ramael M, Nagels J, Heylen H, De Schepper S, Paulussen J, De Maeyer M, Van Haesendonck C: **Detection of SV40 like viral DNA and viral antigens in malignant pleural mesothelioma.** *Eur Respir J* 1999, **14:**1381-1386.

66. Kjærheim K, Røe OD, Waterboer T, Sehr P, Rizk R, Dai HY, Sandeck H, Larsson E, Andersen A, Boffetta P, Pawlita M: **Absence of SV40 antibodies or DNA fragments in prediagnostic**

mesothelioma serum samples. *Int J Cancer* 2007, **120:**2459-2465.

67. Hirvonen A, Mattson K, Karjalainen A, Ollikainen T, Tammilehto L, Hovi T, Vainio H, Pass HI, Di Resta I, Carbone M, Linnainmaa K: **Simian virus 40 (SV40)-like DNA sequences not detectable in finnish mesothelioma patients not exposed to SV40-contaminated polio vaccines.** *Mol Carcinog* 1999, **26:**93-99.

68. Priftakis P, Bogdanovic G, Hjerpe A, Dalianis T: **Presence of simian virus 40 (SV40) is not frequent in Swedish malignant mesotheliomas.** *Anticancer Res* 2002, **22:**1357-1360.

69. Strickler HD: **A multicenter evaluation of assays for detection of SV40 DNA and results in masked mesothelioma specimens.** *Cancer Epidemiol Biomarkers Prev* 2001, **10:**523-532.

70. Manfredi JJ, Dong J, Liu WJ, Resnick-Silverman L, Qiao R, Chahinian P, Saric M, Gibbs AR, Phillips JI, Murray J, Axten CW, Nolan RP, Aaronson SA: **Evidence against a role for SV40 in human mesothelioma.** *Cancer Res* 2005, **65:**2602-2609.

71. Galateau-Salle F (Ed.). **Pathology of Malignant Mesothelioma**. London: Springer Verlag; 2006.

72. Burki T: **Asbestos production increases despite WHO opposition.** *Lancet Oncol* 2009, **10:**846.

73. Kazan-Allen L: **Asbestos and mesothelioma: worldwide trends.** *Lung Cancer* 2005, **49 Suppl 1:**S3-8.

74. Ault JG, Cole RW, Jensen CG, Jensen LC, Bachert LA, Rieder CL: **Behavior of crocidolite asbestos during mitosis in living vertebrate lung epithelial cells.** *Cancer Res* 1995, **55:**792-798.

75. Kamp DW, Graceffa P, Pryor WA, Weitzman SA: **The role of free radicals in asbestos-induced diseases.** *Free Radic Biol Med* 1992, **12:**293-315.

76. Weitzman SA, Graceffa P: **Asbestos catalyzes hydroxyl and superoxide radical generation from hydrogen peroxide.** *Arch Biochem Biophys* 1984, **228:**373-376.

77. Eisenstadt HB: **Asbestos Pleurisy.** *Dis Chest* 1964, **46:**78-81.

78. Martensson G, Hagberg S, Pettersson K, Thiringer G: **Asbestos pleural effusion: a clinical entity.** *Thorax* 1987, **42:**646-651.

79. Faux SP, Houghton CE, Hubbard A, Patrick G: **Increased expression of epidermal growth factor receptor in rat pleural mesothelial cells correlates with carcinogenicity of mineral fibres.** *Carcinogenesis* 2000, **21:**2275-2280.

80. Schäfer M, Werner S: **Cancer as an overhealing wound: an old hypothesis revisited.** *Nat Rev Mol Cell Biol* 2008, **9:**628-638.

81. Boutin C, Rey F, Viallat JR: **Prevention of malignant seeding after invasive diagnostic procedures in patients with pleural mesothelioma. A randomized trial of local radiotherapy.** *Chest* 1995, **108:**754-758.

82. Taguchi T, Jhanwar SC, Siegfried JM, Keller SM, Testa JR: **Recurrent deletions of specific chromosomal sites in 1p, 3p, 6q, and 9p in human malignant mesothelioma.** *Cancer Res* 1993, **53:**4349-4355.

83. Bianchi AB, Mitsunaga SI, Cheng JQ, Klein WM, Jhanwar SC, Seizinger B, Kley N, Klein-Szanto AJ, Testa JR: **High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas.** *Proc Natl Acad Sci U S A* 1995, **92:**10854-10858.

84. Neragi-Miandoab S, Sugarbaker DJ: **Chromosomal deletion in patients with malignant pleural mesothelioma.** *Interact Cardiovasc Thorac Surg* 2009, **9:**42-44.

85. Sekido Y: **Genomic abnormalities and signal transduction dysregulation in malignant mesothelioma cells.** *Cancer Sci* 2009.

86. Kratzke RA, Otterson GA, Lincoln CE, Ewing S, Oie H, Geradts J, Kaye FJ: **Immunohistochemical analysis of the p16INK4 cyclin-dependent kinase inhibitor in malignant mesothelioma.** *J Natl Cancer Inst* 1995, **87:**1870-1875.

87. Xio S, Li D, Vijg J, Sugarbaker DJ, Corson JM, Fletcher JA: **Codeletion of p15 and p16 in primary malignant mesothelioma.** *Oncogene* 1995, **11:**511-515.

88. Mor O, Yaron P, Huszar M, Yellin A, Jakobovitz O, Brok-Simoni F, Rechavi G, Reichert N: **Absence of p53 mutations in malignant mesotheliomas.** *Am J Respir Cell Mol Biol* 1997, **16:**9-13.

89. Segers K, Backhovens H, Singh SK, De Voecht J, Ramael M, Van Broeckhoven C, Van Marck E: **Immunoreactivity for p53 and mdm2 and the**

detection of p53 mutations in human malignant mesothelioma. *Virchows Arch* 1995, **427:**431-436.

90. Metcalf RA, Welsh JA, Bennett WP, Seddon MB, Lehman TA, Pelin K, Linnainmaa K, Tammilehto L, Mattson K, Gerwin BI, et al.: **p53 and Kirsten-ras mutations in human mesothelioma cell lines.** *Cancer Res* 1992, **52:**2610-2615.

91. Pomerantz J, Schreiber-Agus N, Liegeois NJ, Silverman A, Alland L, Chin L, Potes J, Chen K, Orlow I, Lee HW, Cordon-Cardo C, DePinho RA: **The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53.** *Cell* 1998, **92:**713-723.

92. Jagadeeswaran R, Ma PC, Seiwert TY, Jagadeeswaran S, Zumba O, Nallasura V, Ahmed S, Filiberti R, Paganuzzi M, Puntoni R, Kratzke RA, Gordon GJ, Sugarbaker DJ, Bueno R, Janamanchi V, Bindokas VP, Kindler HL, Salgia R: **Functional analysis of c-Met/hepatocyte growth factor pathway in malignant pleural mesothelioma.** *Cancer Res* 2006, **66:**352-361.

93. Destro A, Ceresoli GL, Falleni M, Zucali PA, Morenghi E, Bianchi P, Pellegrini C, Cordani N, Vaira V, Alloisio M, Rizzi A, Bosari S, Roncalli M: **EGFR overexpression in malignant pleural mesothelioma. An immunohistochemical and molecular study with clinico-pathological correlations.** *Lung Cancer* 2006, **51:**207-215.

94. Narasimhan SR, Yang L, Gerwin BI, Broaddus VC: **Resistance of pleural mesothelioma cell lines to apoptosis: relation to expression of Bcl-2 and Bax.** *Am J Physiol* 1998, **275:**L165-171.

95. Soini Y, Kinnula V, Kaarteenaho-Wiik R, Kurttila E, Linnainmaa K, Paakko P: **Apoptosis and expression of apoptosis regulating proteins bcl-2, mcl-1, bcl-X, and bax in malignant mesothelioma.** *Clin Cancer Res* 1999, **5:**3508-3515.

96. Gordon GJ, Appasani K, Parcells JP, Mukhopadhyay NK, Jaklitsch MT, Richards WG, Sugarbaker DJ, Bueno R: **Inhibitor of apoptosis protein-1 promotes tumor cell survival in mesothelioma.** *Carcinogenesis* 2002, **23:**1017-1024.

97. Xia C, Xu Z, Yuan X, Uematsu K, You L, Li K, Li L, McCormick F, Jablons DM: **Induction of apoptosis in mesothelioma cells by antisurvivin oligonucleotides.** *Mol Cancer Ther* 2002, **1:**687-694.

98. Kleinberg L, Lie AK, Florenes VA, Nesland JM, Davidson B: **Expression of inhibitor-of-apoptosis protein family members in malignant mesothelioma.** *Hum Pathol* 2007, **38:**986-994.

99. Zaffaroni N, Costa A, Pennati M, De Marco C, Affini E, Madeo M, Erdas R, Cabras A, Kusamura S, Baratti D, Deraco M, Daidone MG: **Survivin is highly expressed and promotes cell survival in malignant peritoneal mesothelioma.** *Cell Oncol* 2007, **29:**453-466.

100. Hanahan D, Weinberg RA: **The hallmarks of cancer.** *Cell* 2000, **100:**57-70.

101. Gordon GJ, Rockwell GN, Jensen RV, Rheinwald JG, Glickman JN, Aronson JP, Pottorf BJ, Nitz MD, Richards WG, Sugarbaker DJ, Bueno R: **Identification of novel candidate oncogenes and tumor suppressors in malignant pleural mesothelioma using large-scale transcriptional profiling.** *Am J Pathol* 2005, **166:**1827-1840.

102. Kho-Duffin J, Tao LC, Cramer H, Catellier MJ, Irons D, Ng P: **Cytologic diagnosis of malignant mesothelioma, with particular emphasis on the epithelial noncohesive cell type.** *Diagn Cytopathol* 1999, **20:**57-62.

103. Brockstedt U, Gulyas M, Dobra K, Dejmek A, Hjerpe A: **An optimized battery of eight antibodies that can distinguish most cases of epithelial mesothelioma from adenocarcinoma.** *Am J Clin Pathol* 2000, **114:**203-209.

104. Flores-Staino C, Darai-Ramqvist E, Dobra K, Hjerpe A: **Adaptation of a commercial fluorescent in situ hybridization test to the diagnosis of malignant cells in effusions.** *Lung Cancer* 2009.

105. Martensson G, Thylen A, Lindquist U, Hjerpe A: **The sensitivity of hyaluronan analysis of pleural fluid from patients with malignant mesothelioma and a comparison of different methods.** *Cancer* 1994, **73:**1406-1410.

106. Nurminen M, Dejmek A, Martensson G, Thylen A, Hjerpe A: **Clinical utility of liquid-chromatographic analysis of effusions for hyaluronate content.** *Clin Chem* 1994, **40:**777-780.

107. Scherpereel A, Grigoriu B, Conti M, Gey T, Gregoire M, Copin MC, Devos P, Chahine B, Porte H, Lassalle P: **Soluble mesothelin-related peptides in the diagnosis of malignant pleural**

mesothelioma. *Am J Respir Crit Care Med* 2006, **173:**1155-1160.

108. Creaney J, Yeoman D, Naumoff LK, Hof M, Segal A, Musk AW, De Klerk N, Horick N, Skates SJ, Robinson BW: **Soluble mesothelin in effusions: a useful tool for the diagnosis of malignant mesothelioma.** *Thorax* 2007, **62:**569-576.

109. Moschos C, Porfiridis I, Psallidas I, Kollintza A, Stathopoulos GT, Papiris SA, Roussos C, Kalomenidis I: **Osteopontin is upregulated in malignant and inflammatory pleural effusions.** *Respirology* 2009, **14:**716-722.

110. Beyer HL, Geschwindt RD, Glover CL, Tran L, Hellstrom I, Hellstrom KE, Miller MC, Verch T, Allard WJ, Pass HI, Sardesai NY: **MESOMARK: a potential test for malignant pleural mesothelioma.** *Clin Chem* 2007, **53:**666-672.

111. Ellis P, Davies AM, Evans WK, Haynes AE, Lloyd NS: **The use of chemotherapy in patients with advanced malignant pleural mesothelioma: a systematic review and practice guideline.** *J Thorac Oncol* 2006, **1:**591-601.

112. Fennell DA, Gaudino G, O'Byrne KJ, Mutti L, van Meerbeeck J: **Advances in the systemic therapy of malignant pleural mesothelioma.** *Nat Clin Pract Oncol* 2008, **5:**136-147.

113. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, Niyikiza C, Paoletti P: **Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma.** *J Clin Oncol* 2003, **21:**2636-2644.

114. Tomek S, Manegold C: **Chemotherapy for malignant pleural mesothelioma: past results and recent developments.** *Lung Cancer* 2004, **45 suppl 1:**S103-119.

115. Hillerdal G, Sorensen JB, Sundstrom S, Riska H, Vikstrom A, Hjerpe A: **Treatment of malignant pleural mesothelioma with carboplatin, liposomized doxorubicin, and gemcitabine: a phase II study.** *J Thorac Oncol* 2008, **3:**1325-1331.

116. Berg AL, Spitzer JB, Garvin JH, Jr.: **Ototoxic impact of cisplatin in pediatric oncology patients.** *Laryngoscope* 1999, **109:**1806-1814.

117. Rabik CA, Dolan ME: **Molecular mechanisms of resistance and toxicity associated with platinating agents.** *Cancer Treat Rev* 2007, **33:**9-23.

118. Aarne A: **Schwänke über schwerhörige Menschen. Eine vergleichende Untersuchung.** *Folklore Fellows' Communications* 1914, **20**.

119. van Ruth S, Baas P, Zoetmulder FA: **Surgical treatment of malignant pleural mesothelioma: a review.** *Chest* 2003, **123:**551-561.

120. Ceresoli GL, Locati LD, Ferreri AJM, Cozzarini C, Passoni P, Melloni G, Zannini P, Bolognesi A, Villa E: **Therapeutic outcome according to histologic subtype in 121 patients with malignant pleural mesothelioma.** *Lung cancer* 2001, **34:**279-287.

121. Tammilehto L, Maasilta P, Kostiainen S: **Diagnosis and prognostic factors in malignant pleural mesothelioma: a retrospective study of sixty-five patients.** *Respiration* 1992, **59:**129-135.

122. Curran D, Sahmoud T, Therasse P, van Meerbeeck J, Postmus PE, Giaccone G: **Prognostic factors in patients with pleural mesothelioma: the European Organization for Research and Treatment of Cancer experience.** *J Clin Oncol* 1998, **16:**145-152.

123. Antman K, Shemin R, Ryan L, Klegar K, Osteen R, Herman T, Lederman G, Corson J: **Malignant mesothelioma: prognostic variables in a registry of 180 patients, the Dana-Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965-1985.** *J Clin Oncol* 1988, **6:**147-153.

124. Chahinian AP, Pajak TF, Holland JF, Norton L, Ambinder RM, Mandel EM: **Diffuse malignant mesothelioma. Prospective evaluation of 69 patients.** *Ann Intern Med* 1982, **96:**746-755.

125. Boutin C, Rey F, Gouvernet J, Viallat JR, Astoul P, Ledoray V: **Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 2: Prognosis and staging.** *Cancer* 1993, **72:**394-404.

126. Calavrezos A, Koschel G, Husselmann H, Taylessani A, Heilmann HP, Fabel H, Schmoll HJ, Dietrich H, Hain E: **Malignant mesothelioma of the pleura. A prospective therapeutic study of 132 patients from 1981-1985.** *Klin Wochenschr* 1988, **66:**607-613.

127. de Pangher Manzini V, Brollo A, Franceschi S, de Matthaeis M, Talamini R, Bianchi C:

Prognostic factors of malignant mesothelioma of the pleura. *Cancer* 1993, **72:**410-417.

128. Herndon JE, Green MR, Chahinian AP, Corson JM, Suzuki Y, Vogelzang NJ: **Factors predictive of survival among 337 patients with mesothelioma treated between 1984 and 1994 by the Cancer and Leukemia Group B.** *Chest* 1998, **113:**723-731.

129. Berzelius JJ: **Undersökning af en ny Mineral-kropp, funnen i de orenare sorterna af det i Falun tillverkade svaflet.** *Afhandlingar i fysik, kemi och mineralogi* 1818, **6:**42-144.

130. Schrauzer GN: **Selenomethionine: a review of its nutritional significance, metabolism and toxicity.** *J Nutr* 2000, **130:**1653-1656.

131. Brown KM, Arthur JR: **Selenium, selenoproteins and human health: a review.** *Public Health Nutr* 2001, **4:**593-599.

132. Lü J, Jiang C: **Selenium and cancer chemoprevention: hypotheses integrating the actions of selenoproteins and selenium metabolites in epithelial and non-epithelial target cells.** *Antioxid Redox Signal* 2005, **7:**1715-1727.

133. Selenius M, Rundlof AK, Olm E, Fernandes AP, Bjornstedt M: **Selenium and selenoproteins in the treatment and diagnostics of cancer.** *Antioxid Redox Signal* 2009.

134. Ip C: **Lessons from basic research in selenium and cancer prevention.** *J Nutr* 1998, **128:**1845-1854.

135. Ostadalova I, Babicky A, Kopoldova J: **Selenium metabolism in rats after administration of toxic doses of selenite.** *Physiol Bohemoslov* 1988, **37:**159-164.

136. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN: **Characterization of mammalian selenoproteomes.** *Science* 2003, **300:**1439-1443.

137. Reeves MA, Hoffmann PR: **The human selenoproteome: recent insights into functions and regulation.** *Cell Mol Life Sci* 2009, **66:**2457-2478.

138. Morrison DG, Berdan RC, Pauly DF, Turner DS, Oborn CJ, Medina D: **Selenium distribution in mammary epithelial cells reveals its possible mechanism of inhibition of cell growth.** *Anticancer Res* 1988, **8:**51-63.

139. Morrison DG, Medina D: **Distinguishing features of cytotoxic and pharmacological effects of selenite in murine mammary epithelial cells in vitro.** *Toxicol Lett* 1988, **44:**307-314.

140. Morrison DG, Oborn CJ, Medina D: **Selenite distribution in log and confluent growth phase murine mammary epithelial cells.** *Cancer Lett* 1988, **43:**227-236.

141. Morrison DG, Medina D: **Time course of selenite metabolism in confluent cultures of mouse mammary epithelial cells.** *Chem Biol Interact* 1989, **71:**177-186.

142. MacVicar C, Frenkel GD: **Effect of cell density on the inhibition of tumor cell attachment and nucleic acid synthesis by selenite.** *Biol Trace Elem Res* 1993, **39:**139-147.

143. Frisk P, Yaqob A, Nilsson K, Carlsson J, Lindh U: **Uptake and retention of selenite and selenomethionine in cultured K-562 cells.** *Biometals* 2000, **13:**209-215.

144. Morlon H, Fortin C, Adam C, Garnier-Laplace J: **Selenite transport and its inhibition in the unicellular green alga Chlamydomonas reinhardtii.** *Environ Toxicol Chem* 2006, **25:**1408-1417.

145. Lindblow-Kull C, Kull FJ, Shrift A: **Single transporter for sulfate, selenate, and selenite in Escherichia coli K-12.** *J Bacteriol* 1985, **163:**1267-1269.

146. Hudman JF, Glenn AR: **Selenite uptake and incorporation by Selenomonas ruminantium.** *Arch Microbiol* 1984, **140:**252-256.

147. Gharieb MM, Gadd GM: **The kinetics of 75[Se]-selenite uptake by Saccharomyces cerevisiae and the vacuolization response to high concentrations.** *Mycol Res* 2004, **108:**1415-1422.

148. Olm E, Fernandes AP, Hebert C, Rundlof AK, Larsen EH, Danielsson O, Bjornstedt M: **Extracellular thiol-assisted selenium uptake dependent on the x(c)- cystine transporter explains the cancer-specific cytotoxicity of selenite.** *Proc Natl Acad Sci U S A* 2009, **106:**11400-11405.

149. Tarze A, Dauplais M, Grigoras I, Lazard M, Ha-Duong NT, Barbier F, Blanquet S, Plateau P: **Extracellular production of hydrogen selenide accounts for thiol-assisted toxicity of selenite against Saccharomyces cerevisiae.** *J Biol Chem* 2007, **282:**8759-8767.

150. Ganyc D, Self WT: **High affinity selenium uptake in a keratinocyte model.** *FEBS Lett* 2008, **582:**299-304.

151. Batist G, Katki AG, Klecker RW, Jr., Myers CE: **Selenium-induced cytotoxicity of human leukemia cells: interaction with reduced glutathione.** *Cancer Res* 1986, **46:**5482-5485.

152. Caffrey PB, Frenkel GD: **Inhibition of cell colony formation by selenite: involvement of glutathione.** *Mol Pharmacol* 1991, **39:**281-284.

153. Cavalieri RR, Scott KG, Sairenji E: **Selenite (75Se) as a tumor-localizing agent in man.** *J Nucl Med* 1966, **7:**197-208.

154. Cortázar JCF, Velasco JE, Albertino RV, Romeu FP: **Le 75Se-Sélénite dans le diagnostic des tumeurs pulmonaires. Spécificité et problématique biologique.** *J de Radiologie* 1975, **56:**385-388.

155. Jammers W, Ramos M: **Szintigrafische Tumordiagnostik mit 75Se Natriumselenit bei Patienten mit malignen Neoplasien im Thoraxbereich.** *Fortschr Röntgenstr* 1972, **117:**530-537.

156. De Roo MJ: **False-positive 75Se-selenite scan in nonmalignant lesions.** *J Nucl Med* 1974, **15:**622-624.

157. Whitton ID, Houlder AE: **75Se-sodium selenite as a diagnostic aid in the assessment of peripheral pulmonary opacities.** *Thorax* 1979, **34:**102-105.

158. Jereb M, Unge G, Jereb B, Boman G: **Demonstration of malignant tumours in the lungs and mediastinum by means of radionuclear (75Se) scintigraphy.** *Scand J Respir Dis* 1973, **54:**282-289.

159. Esteban J, Lasa D, Perez-Modrego S: **Detection of Cartilaginous Tumors with Selenium 75.** *Radiology* 1965, **85:**149-150.

160. Perez-Modrego S, Esteban J, Lasa D: **Radioactive selenium: a new tracer for detection of cartilaginous tumours.** *Br J Radiol* 1967, **40:**177-180.

161. Kuikka J, Nordman E: **Measurement of 75Se-sodium selenite in the human body.** *Int J Nucl Med Biol* 1978, **5:**30-34.

162. Cater DB, Phillips AF: **Measurement of electrode potentials in living and dead tissues.** *Nature* 1954, **174:**121-123.

163. Cater DB, Phillips AF, Silver IA: **The measurement of oxidation-reduction potentials, pH, and oxygen tension in tumours.** *Proc R Soc Lond B Biol Sci* 1957, **146:**382-399.

164. Jereb M, Falk R, Jereb B, Lindhe C: **Radiation dose to the human body from intravenously administered 75Se-sodium selenite.** *J Nucl Med* 1975, **16:**846-850.

165. Janghorbani M, Kasper LJ, Young VR: **Dynamics of selenite metabolism in young men: studies with the stable isotope tracer method.** *Am J Clin Nutr* 1984, **40:**208-218.

166. Fassbender M, de Villiers D, Nortier M, van der Walt N: **The natBr(p,x) (73,75)Se nuclear processes: a convenient route for the production of radioselenium tracers relevant to amino acid labelling.** *Appl Radiat Isot* 2001, **54:**905-913.

167. Bergmann R, Brust P, Kampf G, Coenen HH, Stocklin G: **Evaluation of radioselenium labeled selenomethionine, a potential tracer for brain protein synthesis by PET.** *Nucl Med Biol* 1995, **22:**475-481.

168. Sandholm M: **The initial fate of a trace amount of intravenously administered selenite.** *Acta Pharmacol Toxicol (Copenh)* 1973, **33:**1-5.

169. Beilstein MA, Butler JA, Whanger PD: **Metabolism of [75Se]selenite by rhesus monkeys.** *J Nutr* 1984, **114:**1501-1509.

170. Beilstein MA, Whanger PD: **Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine.** *J Nutr* 1986, **116:**1711-1719.

171. Ehrlich P: **Address in Pathology. On Chemotherapeutics: Scientific Principles, Methods, and Results.** *Lancet* 1913, **182:**445-451.

172. Nilsonne G, Sun X, Nyström C, Rundlof AK, Fernandes AP, Björnstedt M, Dobra K: **Selenite induces apoptosis in sarcomatoid malignant mesothelioma cells through oxidative stress.** *Free Radic Biol Med* 2006, **41:**874-885.

173. Yan L, Spallholz JE: **Generation of reactive oxygen species from the reaction of selenium compounds with thiols and mammary tumor cells.** *Biochem Pharmacol* 1993, **45:**429-437.

174. Shen HM, Yang CF, Ding WX, Liu J, Ong CN: **Superoxide radical-initiated apoptotic**

signalling pathway in selenite-treated HepG(2) cells: mitochondria serve as the main target. *Free Radic Biol Med* 2001, **30:**9-21.

175. Shen HM, Yang CF, Ong CN: **Sodium selenite-induced oxidative stress and apoptosis in human hepatoma HepG2 cells.** *Int J Cancer* 1999, **81:**820-828.

176. Kim TS, Yun BY, Kim IY: **Induction of the mitochondrial permeability transition by selenium compounds mediated by oxidation of the protein thiol groups and generation of the superoxide.** *Biochem Pharmacol* 2003, **66:**2301-2311.

177. Husbeck B, Bhattacharyya RS, Feldman D, Knox SJ: **Inhibition of androgen receptor signaling by selenite and methylseleninic acid in prostate cancer cells: two distinct mechanisms of action.** *Mol Cancer Ther* 2006, **5:**2078-2085.

178. Husbeck B, Nonn L, Peehl DM, Knox SJ: **Tumor-Selective Killing by Selenite in Patient-Matched Pairs of Normal and Malignant Prostate Cells.** *Prostate* 2006, **66:**218-225.

179. Rudolf E, Rudolf K, Cervinka M: **Selenium activates p53 and p38 pathways and induces caspase-independent cell death in cervical cancer cells.** *Cell Biol Toxicol* 2008, **24:**123-141.

180. Shilo S, Tirosh O: **Selenite activates caspase-independent necrotic cell death in Jurkat T cells and J774.2 macrophages by affecting mitochondrial oxidant generation.** *Antioxid Redox Signal* 2003, **5:**273-279.

181. Kim EH, Sohn S, Kwon HJ, Kim SU, Kim MJ, Lee SJ, Choi KS: **Sodium selenite induces superoxide-mediated mitochondrial damage and subsequent autophagic cell death in malignant glioma cells.** *Cancer Res* 2007, **67:**6314-6324.

182. Hu H, Jiang C, Schuster T, Li GX, Daniel PT, Lu J: **Inorganic selenium sensitizes prostate cancer cells to TRAIL-induced apoptosis through superoxide/p53/Bax-mediated activation of mitochondrial pathway.** *Mol Cancer Ther* 2006, **5:**1873-1882.

183. Kumar S, Björnstedt M, Holmgren A: **Selenite is a substrate for calf thymus thioredoxin reductase and thioredoxin and elicits a large non-stoichiometric oxidation of NADPH in the presence of oxygen.** *Eur J Biochem* 1992, **207:**435-439.

184. Lopez-Lazaro M, Willmore E, Elliott SL, Austin CA: **Selenite induces topoisomerase I and II-DNA complexes in K562 leukemia cells.** *Int J Cancer* 2008, **123:**2217-2221.

185. Park HS, Huh SH, Kim Y, Shim J, Lee SH, Park IS, Jung YK, Kim IY, Choi EJ: **Selenite negatively regulates caspase-3 through a redox mechanism.** *J Biol Chem* 2000, **275:**8487-8491.

186. Park HS, Park E, Kim MS, Ahn K, Kim IY, Choi EJ: **Selenite inhibits the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) through a thiol redox mechanism.** *J Biol Chem* 2000, **275:**2527-2531.

187. Spallholz JE: **Free radical generation by selenium compounds and their prooxidant toxicity.** *Biomed Environ Sci* 1997, **10:**260-270.

188. Fiers W, Beyaert R, Declercq W, Vandenabeele P: **More than one way to die: apoptosis, necrosis and reactive oxygen damage.** *Oncogene* 1999, **18:**7719-7730.

189. Galluzzi L, Aaronson SA, Abrams J, Alnemri ES, Andrews DW, Baehrecke EH, Bazan NG, Blagosklonny MV, Blomgren K, Borner C, Bredesen DE, Brenner C, Castedo M, Cidlowski JA, Ciechanover A, Cohen GM, De Laurenzi V, De Maria R, Deshmukh M, Dynlacht BD, El-Deiry WS, Flavell RA, Fulda S, Garrido C, Golstein P, Gougeon ML, Green DR, Gronemeyer H, Hajnoczky G, Hardwick JM, Hengartner MO, Ichijo H, Jaattela M, Kepp O, Kimchi A, Klionsky DJ, Knight RA, Kornbluth S, Kumar S, Levine B, Lipton SA, Lugli E, Madeo F, Malomi W, Marine JC, Martin SJ, Medema JP, Mehlen P, Melino G, Moll UM, Morselli E, Nagata S, Nicholson DW, Nicotera P, Nunez G, Oren M, Penninger J, Pervaiz S, Peter ME, Piacentini M, Prehn JH, Puthalakath H, Rabinovich GA, Rizzuto R, Rodrigues CM, Rubinsztein DC, Rudel T, Scorrano L, Simon HU, Steller H, Tschopp J, Tsujimoto Y, Vandenabeele P, Vitale I, Vousden KH, Youle RJ, Yuan J, Zhivotovsky B, Kroemer G: **Guidelines for the use and interpretation of assays for monitoring cell death in higher eukaryotes.** *Cell Death Differ* 2009, **16:**1093-1107.

190. Willingham MC: **Cytochemical methods for the detection of apoptosis.** *J Histochem Cytochem* 1999, **47:**1101-1110.

191. Bröker LE, Kruyt FA, Giaccone G: **Cell death independent of caspases: a review.** *Clin Cancer Res* 2005, **11:**3155-3162.

192. Jönsson-Videsäter K, Björkhem-Bergman L, Hossain A, Söderberg A, Eriksson LC, Paul C, Rosen A, Björnstedt M: **Selenite-induced apoptosis in doxorubicin-resistant cells and effects on the thioredoxin system.** *Biochem Pharmacol* 2004, **67:**513-522.

193. Lanfear J, Fleming J, Wu L, Webster G, Harrison PR: **The selenium metabolite selenodiglutathione induces p53 and apoptosis: relevance to the chemopreventive effects of selenium?** *Carcinogenesis* 1994, **15:**1387-1392.

194. Li GX, Hu H, Jiang C, Schuster T, Lu J: **Differential involvement of reactive oxygen species in apoptosis induced by two classes of selenium compounds in human prostate cancer cells.** *Int J Cancer* 2007, **120:**2034-2043.

195. Li S, Zhou Y, Wang R, Zhang H, Dong Y, Ip C: **Selenium sensitizes MCF-7 breast cancer cells to doxorubicin-induced apoptosis through modulation of phospho-Akt and its downstream substrates.** *Mol Cancer Ther* 2007, **6:**1031-1038.

196. Nango R, Terada C, Tsukamoto I: **Jun N-terminal kinase activation and upregulation of p53 and p21(WAF1/CIP1) in selenite-induced apoptosis of regenerating liver.** *Eur J Pharmacol* 2003, **471:**1-8.

197. Shen H, Yang C, Liu J, Ong C: **Dual role of glutathione in selenite-induced oxidative stress and apoptosis in human hepatoma cells.** *Free Radic Biol Med* 2000, **28:**1115-1124.

198. Xiao R, Qiao JT, Zhao HF, Liang J, Yu HL, Liu J, Guo AM, Wang W: **Sodium selenite induces apoptosis in cultured cortical neurons with special concomitant changes in expression of the apoptosis-related genes.** *Neurotoxicology* 2006, **27:**478-484.

199. Zhao R, Xiang N, Domann FE, Zhong W: **Expression of p53 Enhances Selenite-Induced Superoxide Production and Apoptosis in Human Prostate Cancer Cells.** *Cancer res* 2006, **66:**2296-2304.

200. Chung YW, Kim TS, Lee SY, Lee SH, Choi Y, Kim N, Min BM, Jeong DW, Kim IY: **Selenite-induced apoptosis of osteoclasts mediated by the mitochondrial pathway.** *Toxicol Lett* 2006, **160:**143-150.

201. Jiang C, Hu H, Malewicz B, Wang Z, Lu J: **Selenite-induced p53 Ser-15 phosphorylation and caspase-mediated apoptosis in LNCaP human prostate cancer cells.** *Mol Cancer Ther* 2004, **3:**877-884.

202. Jiang C, Wang Z, Ganther H, Lu J: **Distinct effects of methylseleninic acid versus selenite on apoptosis, cell cycle, and protein kinase pathways in DU145 human prostate cancer cells.** *Mol Cancer Ther* 2002, **1:**1059-1066.

203. Guan L, Huang F, Li Z, Han B, Jiang Q, Ren Y, Yang Y, Xu C: **P53 transcription-independent activity mediates selenite-induced acute promyelocytic leukemia NB4 cell apoptosis.** *BMB Rep* 2008, **41:**745-750.

204. Guan L, Han B, Li J, Li Z, Huang F, Yang Y, Xu C: **Exposure of human leukemia NB4 cells to increasing concentrations of selenite switches the signaling from pro-survival to pro-apoptosis.** *Ann Hematol* 2009, **doi:10.1007/s00277-008-0676-4**.

205. Smith ML, Lancia JK, Mercer TI, Ip C: **Selenium compounds regulate p53 by common and distinctive mechanisms.** *Anticancer Res* 2004, **24:**1401-1408.

206. Chen YR, Tan TH: **The c-Jun N-terminal kinase pathway and apoptotic signaling (review).** *Int J Oncol* 2000, **16:**651-662.

207. Hazzalin CA, Mahadevan LC: **MAPK-regulated transcription: a continuously variable gene switch?** *Nat Rev Mol Cell Biol* 2002, **3:**30-40.

208. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH: **Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions.** *Endocr Rev* 2001, **22:**153-183.

209. Ren Y, Huang F, Liu Y, Yang Y, Jiang Q, Xu C: **Autophagy inhibition through PI3K/Akt increases apoptosis by sodium selenite in NB4 cells.** *BMB Rep* 2009, **42:**599-604.

210. Kahlos K, Soini Y, Saily M, Koistinen P, Kakko S, Paakko P, Holmgren A, Kinnula VL: **Up-regulation of thioredoxin and thioredoxin reductase in human malignant pleural mesothelioma.** *Int J Cancer* 2001, **95:**198-204.

211. Mohr S, Bottin MC, Lannes B, Neuville A, Bellocq JP, Keith G, Rihn BH: **Microdissection, mRNA amplification and microarray: a study of pleural mesothelial and malignant mesothelioma cells.** *Biochimie* 2004, **86:**13-19.

212. Rihn BH, Mohr S, McDowell SA, Binet S, Loubinoux J, Galateau F, Keith G, Leikauf GD:

Differential gene expression in mesothelioma. *FEBS letters* 2000, **480:**95-100.

213. Nilsonne G, Stein A, Rundlöf A-K, Fernandes AP, Björnstedt M, Hjerpe A, Dobra K: **Targeting thioredoxin reductase – a possible new treatment for malignant mesothelioma. .** *Lung cancer* 2006, **54:**S52.

214. Chaudiere J, Courtin O, Leclaire J: **Glutathione oxidase activity of selenocystamine: a mechanistic study.** *Arch Biochem Biophys* 1992, **296:**328-336.

215. Madeja Z, Sroka J, Nystrom C, Bjorkhem-Bergman L, Nordman T, Damdimopoulos A, Nalvarte I, Eriksson LC, Spyrou G, Olsson JM, Bjornstedt M: **The role of thioredoxin reductase activity in selenium-induced cytotoxicity.** *Biochem Pharmacol* 2005, **69:**1765-1772.

216. Rigobello MP, Gandin V, Folda A, Rundlof AK, Fernandes AP, Bindoli A, Marzano C, Bjornstedt M: **Treatment of human cancer cells with selenite or tellurite in combination with auranofin enhances cell death due to redox shift.** *Free Radic Biol Med* 2009, **47:**710-721.

217. Yan L, Yee JA, Boylan LM, Spallholz JE: **Effect of selenium compounds and thiols on human mammary tumor cells.** *Biol Trace Elem Res* 1991, **30:**145-162.

218. Ranawat P, Bansal MP: **Decreased glutathione levels potentiate the apoptotic efficacy of selenium: possible involvement of p38 and JNK MAPKs--in vitro studies.** *Mol Cell Biochem* 2008, **309:**21-32.

219. Kiessling R, Klein E, Wigzell H: **"Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype.** *Eur J Immunol* 1975, **5:**112-117.

220. Malmberg KJ, Bryceson YT, Carlsten M, Andersson S, Bjorklund A, Bjorkstrom NK, Baumann BC, Fauriat C, Alici E, Dilber MS, Ljunggren HG: **NK cell-mediated targeting of human cancer and possibilities for new means of immunotherapy.** *Cancer Immunol Immunother* 2008, **57:**1541-1552.

221. Papamichail M, Perez SA, Gritzapis AD, Baxevanis CN: **Natural killer lymphocytes: biology, development, and function.** *Cancer Immunol Immunother* 2004, **53:**176-186.

222. Chavez-Galan L, Arenas-Del Angel MC, Zenteno E, Chavez R, Lascurain R: **Cell death mechanisms induced by cytotoxic lymphocytes.** *Cell Mol Immunol* 2009, **6:**15-25.

223. Schröterová L, Králová V, Vorácová A, Hasková P, Rudolf E, Cervinka M: **Antiproliferative effects of selenium compounds in colon cancer cells: Comparison of different cytotoxicity assays.** *Toxicol In Vitro* 2009.

224. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C: **A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V.** *J Immunol Methods* 1995, **184:**39-51.

225. Keston AS, Brandt R: **The Fluorometric Analysis of Ultramicro Quantities of Hydrogen Peroxide.** *Anal Biochem* 1965, **11:**1-5.

226. Hu ML: **Measurement of protein thiol groups and glutathione in plasma.** *Methods Enzymol* 1994, **233:**380-385.

227. Zellos LS, Sugarbaker DJ: **Diffuse malignant mesothelioma of the pleural space and its management.** *Oncology (Williston Park)* 2002, **16:**907-913; discussion 916-907, 919-920, 925.

228. Urig S, Becker K: **On the potential of thioredoxin reductase inhibitors for cancer therapy.** *Semin Cancer Biol* 2006, **16:**452-465.

229. Arner ES, Holmgren A: **The thioredoxin system in cancer.** *Semin Cancer Biol* 2006, **16:**420-426.

230. Ren X, Bjornstedt M, Shen B, Ericson ML, Holmgren A: **Mutagenesis of structural half-cystine residues in human thioredoxin and effects on the regulation of activity by selenodiglutathione.** *Biochemistry* 1993, **32:**9701-9708.

231. Forceville X: **Effects of high doses of selenium, as sodium selenite, in septic shock patients a placebo-controlled, randomized, double-blind, multi-center phase II study--selenium and sepsis.** *J Trace Elem Med Biol* 2007, **21 Suppl 1:**62-65.

232. Kamble P, Mohsin N, Jha A, Date A, Upadhaya A, Mohammad E, Khalil M, Pakkyara A, Budruddin M: **Selenium intoxication with selenite broth resulting in acute renal failure and severe gastritis.** *Saudi J Kidney Dis Transpl* 2009, **20:**106-111.

233. Lopez-Rios F, Chuai S, Flores R, Shimizu S, Ohno T, Wakahara K, Illei PB, Hussain S, Krug L, Zakowski MF, Rusch V, Olshen AB, Ladanyi M: **Global gene expression profiling of pleural mesotheliomas: overexpression of aurora kinases and P16/CDKN2A deletion as prognostic factors and critical evaluation of microarray-based prognostic prediction.** *Cancer Res* 2006, **66:**2970-2979.

234. Sun X, Dobra K, Björnstedt M, Hjerpe A: **Upregulation of 9 genes, including that for thioredoxin, during epithelial differentiation of mesothelioma cells.** *Differentiation* 2000, **66:**181-188.

235. Sun X, Wei L, Liden J, Hui G, Dahlman-Wright K, Hjerpe A, Dobra K: **Molecular characterization of tumour heterogeneity and malignant mesothelioma cell differentiation by gene profiling.** *J Pathol* 2005, **207:**91-101.

236. Sun X, Gulyas M, Hjerpe A, Dobra K: **Proteasome inhibitor PSI induces apoptosis in human mesothelioma cells.** *Cancer Lett* 2006, **232:**161-169.

237. Blumenthal RD: **An overview of chemosensitivity testing.** *Methods Mol Med* 2005, **110:**3-18.

238. Overholtzer M, Mailleux AA, Mouneimne G, Normand G, Schnitt SJ, King RW, Cibas ES, Brugge JS: **A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion.** *Cell* 2007, **131:**966-979.

239. Pereira TC, Saad RS, Liu Y, Silverman JF: **The diagnosis of malignancy in effusion cytology: a pattern recognition approach.** *Adv Anat Pathol* 2006, **13:**174-184.

240. Bode AM, Dong Z: **The functional contrariety of JNK.** *Mol Carcinog* 2007, **46:**591-598.

241. Bradham C, McClay DR: **p38 MAPK in development and cancer.** *Cell Cycle* 2006, **5:**824-828.

242. Mai Z, Liu H: **Boolean network-based analysis of the apoptosis network: irreversible apoptosis and stable surviving.** *J Theor Biol* 2009, **259:**760-769.