

Alpha-fetoprotein and Its Receptor in Fixing the Cancer Brakes

Alpha-fetoprotein and Its Receptor in Fixing the Cancer Brakes

By

Vladimir N. Pak

**Cambridge
Scholars
Publishing**



Alpha-fetoprotein and Its Receptor in Fixing the Cancer Brakes

By Vladimir N. Pak

This book first published 2021

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

Copyright © 2021 by Vladimir N. Pak

All rights for this book reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the copyright owner.

ISBN (10): 1-5275-6716-8

ISBN (13): 978-1-5275-6716-0

**Alpha-fetoprotein:
The Protein You'll Never Give Up**

*Of all the human proteins I see
The most extraordinary one is AFP.
In many assays that men have devised
Its presence indicates birth or demise.
Its functions, which first may appear insipid,
In context, it becomes most important and vivid.
Who said, "AFP over-runneeth its cup"?
I say it's a protein you'll never give up!*

—Vladimir N. Pak, 2019



CONTENTS

List of Illustrations	x
List of Tables.....	xiii
Foreword and Author Biography.....	xiv
Acknowledgements	xvi
List of Abbreviations.....	xvii
Introduction	1
Chapter 1	3
Indulgence Mutations in Cancer Cells	
1.1. Mutations are inevitable.....	3
1.2. Apoptosis controls mutations from the inside.....	4
1.3. The immune system controls mutations from the outside.....	6
1.4. Indulgence mutations	7
1.5. Re-expressions of oncofetal genes as indulgence mutations.....	9
Chapter 2	14
A Tumor, the Embryo's Devil Mutant	
2.1. The interaction between the embryo and the mother's immunity...	14
2.2. Immune tolerance in cancer is similar to that during pregnancy..	17
Chapter 3	20
AFP as a Shuttle Delivery Protein	
3.1. Alpha-fetoprotein characteristics and its physiological role	20
3.2. AFP properties depend on its purification.....	24
3.3. AFP as a shuttle transport protein and a nano-container.....	27
3.4. AFP-binding receptors	30

Chapter 4	33
Toxins for Cancer Therapy	
4.1. Apoptosis as a key process to be fixed by cancer therapy	34
4.2. AFP-binding toxins	37
4.3. Aprotinin	38
4.4. The cell wall of <i>Lactobacillus bulgaricus</i>	41
4.5. Saponins and soaps	42
4.6. Organelles-targeted toxins	45
4.7. Embryo toxins or teratogens	46
Chapter 5	48
Cancer Immunotherapy	
5.1. Innate immunity stimulation to mount an aggressive response against the tumor	48
5.2. Immune checkpoint inhibitors	49
5.3. CAR T cells	51
5.4. CAR NK cells	53
5.5. MDSC major role in immune suppression	54
5.6. MDSC as a “magic target” of immunotherapy	59
Chapter 6	67
AFP and Autoimmune Diseases	
6.1. MDSCs play an important role in regulation of some pathological conditions	67
6.2. Alfetin—the first medicine based on AFP	71
6.3. AFP and neonatal Fc receptor in autoimmune diseases	73
Chapter 7	75
AFPR-Targeted Cancer Therapy	
7.1. Antibodies to AFPR	75
7.2. AFP+toxin covalent conjugates	77
7.3. AFP+toxin non-covalent complexes	79
7.4. The purification of proteins for injections	82
7.5. Reducin	84
7.6. Embryo extracts	88
7.7. Porcine AFP+toxins complexes	89
7.8. The results of the clinical implementation of Aimpila	101
7.9. The possible mechanism for the peroral forms of AFP complexes absorption and action	107
7.10. AFP-mediated potentiation of traditional medicines	113

Chapter 8	125
Treatment for Metastases	
Chapter 9	129
AFP Forever: It's a Protein You'll Never Give Up!	
Appendix	132
References	139

LIST OF ILLUSTRATIONS

- Figure 1-1. Protein p53 controls the DNA mutation state and directs the cell to repair and duplication (left) or to death (right).
- Figure 1-2. MDSCs beneficial impact on pregnancy and tumor tolerance. Adapted from (Ostrand-Rosenberg and Fenselau 2018).
- Figure 2-1. MDSC-mediated mechanisms of immunosuppression (MDSCs R&D Systems poster 2019, with permission).
- Figure 3-1. AFP synthesis during different periods of life. In healthy adults, AFP is synthesized in low amounts. The question is: can AFP at high levels modulate diseases in adults? Adapted from (Abelev 1971).
- Figure 3-2. The overall architecture and domain organization of the AFP 3D model structure. (A) The three-domain organization of the AFP. (B) AFP surface with a tunnel and cavity. This pocket can fit 1-2 hydrophobic molecules. (C) A groove originating from the pit. From (Moldogazieva et al. 2020), with permission.
- Figure 4-1. Intrinsic and extrinsic apoptosis pathways and proliferation-associated chemotherapy targets rely on p53 and apoptosis in the subsequent steps. Cytotoxic T lymphocyte or the death receptor of the extrinsic pathway has a shortcut to apoptosis (Pak 2008). Illustration © 2008 AXS Biomedical Animation Studio used with permission.
- Figure 4-2 [A]. HPLC of aprotinin-containing registered drugs, Ingitril and Contrical. Aprotinin identification was completed according to its time of elution (peak # 1, time of elution = 16,4 min), and wavelength D206/D280 identification ratio = 41,6. Econova Milichrom A-02 (Novosibirsk, Russian Federation) liquid chromatography with C₁₈ (ProntoSil) column and standard gradient 0.2 M LiClO₄+0.005 M HClO₄:100% acetonitrile used according to the manufacturer's manual instructions; similar to those described in (Baram et al. 1983; Azarova Baram, and Goldberg 2006).
- Figure 4-2 [B]. Injectable drug Ingitril manufactured by the Novosibirsk Plant of Medical Preparations and a Diploma for the finalists of the 100 best products of the Russian Federation (1999).
- Figure 4-3. Soap/saponin can lyse bacterium (A), or organelle membranes (B) delivered by AFP. Organelle membrane damage leads to the apoptosis of the eukaryotic cell.

Figure 5-1. Small numbers of regulatory MDSCs and T-regs suppress numerous subordinate executive CTLs and NK cells. AFP-mediated nutrition supply enhances immune suppression.

Figure 7-1. 12% polyacrylamide gel electrophoresis of the two 55 kDa Hyaluronidase preparations. Columns: BSA, 67 kDa bovine serum albumin marker; 1, Lydasum with a high BSA content; 2, Our Lydasum (55 kDa HY) with a low BSA content.

Figure 7-2. Reducin was a combination of the two injectable drugs, Alfetin and amphotericin B, registered in the Russian Federation.

Figure 7-3. HPLC of 95% pure rAFP and thapsigargin non-covalent complex (1:2 molar ratio). Eight wavelengths are used for absorption detection. Peak rAFP eluted at 13 minutes; TG peak eluted at 17.7 minutes. Chromatography conditions were similar to those described in Figure 4-2.

Figure 7-4. 12% PAAG gel electrophoresis of porcine embryo's blood pAFP-containing fractions. Column Standard is the protein molecular weights controls; Columns A and C are the blood protein fractions used for pAFP+toxin preparations. The preparation contains pAFP (70 kDa), albumin (67 kDa), and other minor impurities.

Figure 7-5. Glycoside atractyloside hits mitochondria, so neither the p53 conditions nor the Bcl-2/Bax imbalance prevents apoptosis.

Figure 7-6. ATR oil suspension improves the survival of mice with Ca-755 breast adenocarcinoma cells inoculated subcutaneously. Control mice gavage with water or oil.

Figure 7-7. HPLC of porcine AFP+Atractyloside complex. Atractyloside elutes at 7 min., albumin at 12,5 min., pAFP at 13.5 min. Chromatography conditions were the same as described in Fig. 4-2 [A].

Figure 7-8. The growth of a P-388 lymphocytic leukemia cell line in DBA2 mice treated daily as indicated beginning one-day post-inoculation. 20,000 leukemia cells were inoculated subcutaneously into the sides of DBA2 mice. All animals survived post-inoculation. Starting the next day after the leukemia cell inoculation, each group consisting of 10 mice received daily 0.2 ml oral dosages of the indicated treatment in oil delivery vehicles. 1, preparation A: pAFP+ATR in oil; 2, preparation T: pAFP+TG in oil; 3, preparation S: spleen extract in oil; 4, preparations A+S; and 5, preparation T+S. Control 1, water and Control 2, oil.

Figure 7-9. Improved survival of tumor-bearing mice after daily intake of preparations 1–5. Experiment conditions and samples description, as in Figure 7-8 and the text.

- Figure 7-10. Liver Computed Tomography scans before and after Aimpila treatments. The arrows show locations and reductions of the metastases (Pak, Molchanov, and Vincent 2007).
- Figure 7-11. Hypothetical AFP+toxin complex transported from the gut to the lymphatic system due to the FcRn enterocyte receptor (R). In the lamina propria and lymph node, DC or other AFP-binding regulatory immune cells (RIC) absorb the complex through specific receptor(s) (R). After complex dissociation, the toxin kills the cells. Adapted from (Pyzik et al. 2015).
- Figure 7-12. HPLC of pAFP+ajoene complex. The complex consists of ajoene, albumin, pAFP, and impurities. Chromatography conditions were similar to those described in Figure 4-2 [A].
- Figure 7-13. The growth of P-388 leukemia cells after a daily oral gavage of 0.2 ml of an oily suspension of preparations C1, C2, A, D, and T, starting the second day after 20, 000 tumor cells/mouse inoculation. The number of mice that survived is in the brackets.
- Figure 9-1. Immune response modulation with AFP-ligand complexes. (A) AFP with medication binds MDSC and generate immune tolerance; (B) AFP with toxin binds MDSC and promotes cancer immunotherapy.

LIST OF TABLES

Table 3-1. AFP receptors are shed from the cell membranes and detected in the serum of patients with different cancers. Adapted from (Moro et al. 2005).

Table 7-1. Comparison of AFP, recombinant AFP (rAFP), and porcine AFP (pAFP) properties.

Table 7-2. Mice survival rates and tumor sizes on the 24th day of the experiment.

Table 7-3. Comparison of Abraxane and Aimpila properties.

Table 7-4. The numbers of mice in 5 groups and a description of their treatments.

FOREWORD AND AUTHOR BIOGRAPHY

My name is Vladimir N. Pak. I graduated from the Novosibirsk State University and I earned my Ph.D. degree at the Moscow Institute of Bioorganic Chemistry. From December 1982 until March 1994, I worked at the All-Union Institute of Molecular Biology (known today as “SRC VB VECTOR”). I was then the head of biotechnological departments in “Medical Technologies” and the “Novosibirsk Plant of Medical Preparations”. In 2002, I moved to Canada. Since then, my work has mostly focused on food supplements for humans and animals.

Over the course of my 35+ years of post-doctoral experience in genetic engineering, virology, immunology, biotechnology, pharmaceutical industry, and drug development, I have authored nine patents related to active pharmaceutical ingredients and the manufacturing of medicines. Among other substances and technologies, I have invented an anti-cancer medicine for cancer cells-targeted chemotherapy that also turned out to be a myeloid-derived suppressor cells (MDSCs)-targeted immunotherapy. This medicine can be used in the form of injectable drugs or peroral supplements. During my scientific and industrial career in Russia and in Canada, I modernized existing and created several new technologies, which are used by different companies to manufacture active pharmaceutical ingredients for drugs and drug-candidates:

- Hyaluronidase (Lydazum): an enzyme that dissolves hyaluronic acid in scars; assists fertilization and facilitates the absorption of other injectable drugs. This technology has been patented in the Russian Federation and transferred to a pharmaceutical manufacturing company.
- Aprotinin (Ingitritil): a basic trypsin inhibitor that prevents unwanted proteolysis in the blood and the pancreas. This technology has been patented in the Russian Federation and transferred to a pharmaceutical manufacturing company.
- Reducin: an injectable drug-candidate for the cancer targeted chemotherapy. Based on a complex of the human alpha-fetoprotein (AFP) with a toxin. This technology has been patented in the Russian Federation and in the USA.

- A dietary supplement with an extract of porcine embryonic protein. Based on the method of butanol extraction using my original know-how. To date, the technology has not yet been transferred; the substance for this dietary supplement was manufactured in Canada until 2008.
- Anti-cancer drug-candidates for peroral administration. Several compositions of purified porcine AFP with different toxins for the treatment of cancer have been patented in the USA and in several European countries. This technology has not been transferred. The last samples of these compositions were prepared in 2012.
- RegenerEQ: a gastrointestinal regenerator for horses. This technology belongs to the manufacturer.
- Litis: a dietary supplement for human digestive health. This technology belongs to the manufacturer.

Several companies have used my technologies to manufacture commercial products. Some other developments have not yet been tested in production.

The most exciting and significant outcomes of my research are described in this book and revolve around a unique protein—AFP. In particular, I have investigated its role in embryo- and oncogenesis, as well as have studied its applications in cancer treatment.

ACKNOWLEDGEMENTS

I genuinely admire the talent and dedication of Dr. Gerald J. Mizejewski, a man who has devoted his life to studying the AFP. His work has played a pivotal role in my appreciation of this unique protein.

I am thankful to my Russian friends and colleagues S. Reshetnikov and V. Starikov, who have helped me to apply my knowledge and experience to create new technologies, drugs, and medicines.

I am also grateful to the following:

- S. Nikonov MD, Ph.D. for the discussions and consultations about the anti-cancer activity of Reducin.
- Dr. B. Korczak, Dr. W. Hill, Dr. L. Kim, and Dr. V. Litinski—MaRS Discovery District reviewers and mentors.
- Dr. A. Wainson and Dr. V. Mesharikova for conducting pre-clinical safety and efficacy trials of my compositions in their laboratory in the Russian Federation (Chapter 7.7).
- O. Molchanov MD, Ph.D. for organizing and performing the clinical trials of my drug-candidates in the Russian Federation (Chapter 7.8).
- M. Vincent MD, Ph.D. for helpful discussions of the results of the pre-clinical and clinical trials of Aimpila, and for the co-presentation of these results at ASCO 2007 in Chicago, USA.
- Dr. N. Belyaev for illuminating discussions about AFP interaction with natural suppressor cells (known today as MDSCs), and the immune tolerance reversal in cancer.
- Dr. R. Karapetian, Dr. S. Leonov, and N. Shevkun (ChemDiv Inc., Moscow, Russia) for investigating the bioavailability of the peroral pAFP+toxin complex and for confirming that it does not penetrate through the gastrointestinal tract into the blood, which is a significant scientific result related to pAFP+toxin complexes.
- My family and friends who have helped me accomplish this job.

LIST OF ABBREVIATIONS

AA*	Arachidonic acid
AD	Autoimmune disease
AFC	Antibody-forming cell
AFP	Alpha-fetoprotein
rAFP	Human Recombinant AFP
pAFP	Porcine AFP
AFPR	AFP receptor
ATR*	Atractyloside
ATRA*	All-trans-retinoic acid
BA*	Betulinic acid
Bcl2/Bax	Proteins localized to the outer membrane of mitochondria
CAF	Cancer-associated fibroblast
CCR5	C-C chemokine receptor type 5
CEA	Carcinoembryonic antigen
CTL	Cytolytic T lymphocyte
CTLA-4	CTL-associated protein 4
DC	Dendritic cell
DHA*	Docosahexaenoic acid
DES*	Diethylstilbestrol
Dox*	Doxorubicin
EPA*	Eicosapentaenoic acid
EPR	Endoplasmic reticulum
FcRn	Neonatal Fc receptor
5-FU	5-fluorouracil
GI	Gastrointestinal
GI ₅₀	The concentration for 50% of the maximal inhibition of cell proliferation
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HCC	Hepatocellular carcinoma
hCG	Human chorionic gonadotropin
HPLC	High performance liquid chromatography
HSC	Hematopoietic stem cell
IBD	Inflammatory bowel disease
ICI	Immune checkpoint inhibitor
LD ₅₀	Median lethal dose

MDSC	Myeloid-derived suppressor cell
MHC I	Major Histocompatibility Complex class I (or HLA in humans)
MS	Multiple sclerosis
MTX*	Methotrexate
NK	Natural Killer
p53	Protein 53
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death ligand 1
PUFA*	Polyunsaturated fatty acid
RA	Rheumatoid arthritis
TAM	Tumor-associated macrophage
TG*	Thapsigargin
TGF- β	Tumor growth factor beta
T-reg	Regulatory T cell
TME	Tumor microenvironment
VEGF	Vascular endothelial growth factor
β 2M	Beta 2 microglobulin

* The structural formulas and molecular weights of the agents cited in the text can be found in the Appendix.

INTRODUCTION

Do not reinvent the wheel—fix the brakes.

Cancer is relentless. It kills millions of people every year and represents one of the most significant health challenges for humanity. Every sixth death in the world is due to cancer, making it one of the leading causes of death yielding only to cardiovascular diseases. Is there anything we can do about it?

Perhaps, we should listen to Hippocrates: *“The natural healing force within each of us is the greatest force in getting well.”*

Cancer is a collective name for many different diseases and all of them are characterized by the uncontrolled proliferation and cancerous transformation of abnormal cells. Many chemotherapies in cancer treatment target cellular proliferation mechanisms, damaging them in different ways. However, clinical practice shows that this is not enough, as the proliferation control inside the cancer cells is broken. The external control mechanism—our immunity—also fails.

In this book, I will focus on the natural cancer controls (brakes) that serve the majority of us and on the approaches to fix the damaged brakes in cancer patients with the help of alpha-fetoprotein (AFP).

AFP is an oncofetal protein, i.e., it activates in developing (fetal) as well as in growing cancer (onco) cells and delivers nutrients through specific cell receptors. Its role is essential during normal development but, after birth, AFP’s detection level in healthy individuals is miniscule. Another re-activation may happen in the case of a disease. Due to such properties, AFP may enable the targeted delivery of toxins instead of nutrients into cancer cells.

There can be various targets for toxins inside a cell. Unlike proliferation-targeting toxins, in our approach we have selected and used such substances that can fix the control of proliferation which is damaged in cancer cells. After that, apoptosis (programmed cell death) destroys abnormal cells naturally without detrimental side-effects.

The second cancer brake—our immunity—can be fixed with immunotherapy. Modern immunotherapies that employ T or B cells have only demonstrated efficacy in a fraction of patients. Another promising approach is based on monocytes which regulate T and B cells. A small monocyte subpopulation—myeloid-derived suppressor cells (MDSCs)—prevents fetus rejection but also generates a profound immune tolerance in disease. Physiologic factors, such as pregnancy, stress, and age, elevate MDSCs levels. As with embryo and cancer cells, the activity of MDSCs is stimulated by the AFP-mediated supply of nutrients. Alternatively, AFP can accurately deliver toxins instead of nutrients and destroy MDSCs and cancer cells. Among many types of immunotherapy, the depletion of MDSCs is the preferable mechanism since it unleashes both innate and adaptive immunity in order to erase cancer cells.

In our work, we have demonstrated that AFP+toxin drugs can act as “two in one” by combining a potent immunotherapy with a cancer cells-targeted chemotherapy. I am sure that the progress in understanding and applying AFP will eventually lead to effective anti-cancer drugs. Such efforts will be facilitated by the increasing availability of recombinant AFP (rAFP) which has recently been used instead of the natural protein. RAFPs (or its fragments) are used in anti-cancer AFP+toxin combinations, which appear in multiple patents.

In addition to the human protein used in our earlier patent, I have investigated porcine AFP. Surprisingly, its complexes with selected food ingredients or spices taken as supplements have demonstrated anti-cancer properties in mice models and humans.

AFP potentiates the efficacy of certain drugs/agents and their combinations can even act as prophylactics for cancer and metastases. My belief is that the urgent problem of metastases can be elegantly solved with AFP and, importantly, rather soon.

Finally, AFP has demonstrated its potential in treating autoimmune diseases (ADs). Considering the ability of AFP to bind and supply MDSCs with selected ligands, we can expect that AFP-based products may open up a new era of drugs and medicines for treating multiple MDSC-dependent conditions.

My dream is that AFP and AFP+drugs complexes will be registered, and people in need will enjoy the benefits of safe and effective treatments such as occurred with some of my previous drugs and remedies.

CHAPTER 1

INDULGENCE MUTATIONS IN CANCER CELLS

1.1. Mutations are inevitable

Every day dozens of billions of cells in our body die, and new cells appear via duplication. Each division inevitably generates mutations in the genome, and there is not much we can do about it. Mutations are random and all genes in any position in the genome can mutate, which can give them an advantage so that they will start proliferating, growing, and building a body of cancer. Accumulation of mutations in a healthy cell can lead to its transformation into a cancerous one. Cancer starts when the cells collect a combination of genetic mutations that allows them to grow out of control.

Moreover, cancer-associated mutations may pre-exist and propagate from ancestral clones. Genetic clones that contribute to the disease already possess characteristics such as therapeutic tolerance. These clones can sometimes be isolated years before the disease (Waanders et al. 2020). Specific inherited genetic mutations inhibit the body's ability to safeguard and repair DNA and thus increase the risk of cancer. For example, mutations in BRCA1 and BRCA2 tumor suppressor genes that help repair DNA represent known genetic risk factors for breast and epithelial ovarian cancer (Nacson et al. 2020).

The mutations leading to cancer often arise due to exposure to UV light, tobacco, smoke, or carcinogenic chemicals. Mutations can lead to the activation or deactivation of proteins and dysregulation in a variety of cellular processes.

Healthy living cannot prevent cancer since random luck plays quite a significant role here. Hereditary mutations are responsible for 5%, environment factors for 29%, and mutations during the DNA replication for 66% of cancers (Tomasetti and Vogelstein 2015). So, the probability of developing cancer gradually increases as one becomes older.

At the time of diagnosis, cancer cells can contain thousands of mutations and can harbor up to one million mutations as a product of further neoplastic evolution. This is why the primary strategy of modern oncology is based on earlier diagnostics when treatment is more accessible and can be the most effective.

Out of hundreds of point mutations identified in a patient, only a few are responsible for cellular transformations leading to cancer and therefore drive the disease. Such “driver” mutations give cancer cells a selective advantage leading to their proliferation, additional functionality, and active division. The recurrence of a mutation in patients remains one of the most reliable markers of its driver status. Mutations that alter the function of driver genes are the key players in cancer progression (Chen et al. 2017). On average, up to 10 mutations are needed for cancer to emerge, but the number of driving mutations varies considerably across different cancer types (Martincorena et al. 2017). Cancer-driving mutations are at the root of cancers, and they are more likely to occur than “passenger” mutations. The latter, which constitutes the majority of all observed mutations, have mostly neutral functional impacts and are unlikely to experience the selection pressure (Brown et al. 2019).

1.2. Apoptosis controls mutations from the inside

Cancer occurs when damaged cells grow, divide, and spread abnormally instead of self-destructing as they should. Only cells that accumulate necessary mutations survive. Irradiation and chemotherapy can damage the genome of cancer cells and eventually trigger the existing built-in mechanisms that are supposed to prevent the disease. Most of the time, cells themselves detect and repair mutations or damage in the DNA. If a cell is damaged and cannot repair itself, it usually undergoes the so-called programmed cell death, or apoptosis. During the DNA replication and cell division, apoptosis erases cells with non-repairable mutations. At the same time, some “secondary cancers,” which are completely separate from the primary disease, may appear after aggressive cancer treatments.

Certain mutations may prevent apoptosis; they appear to be obligatory in the majority of cancer cells. For example, mutations in the gene that codes for the protein 53 (p53). Discovered in 1979, oncogene p53 is involved in the DNA repair system and is the key player in apoptosis, which is often broken in cancer cells (Abeglen et al. 2015) (Fig. 1-1).

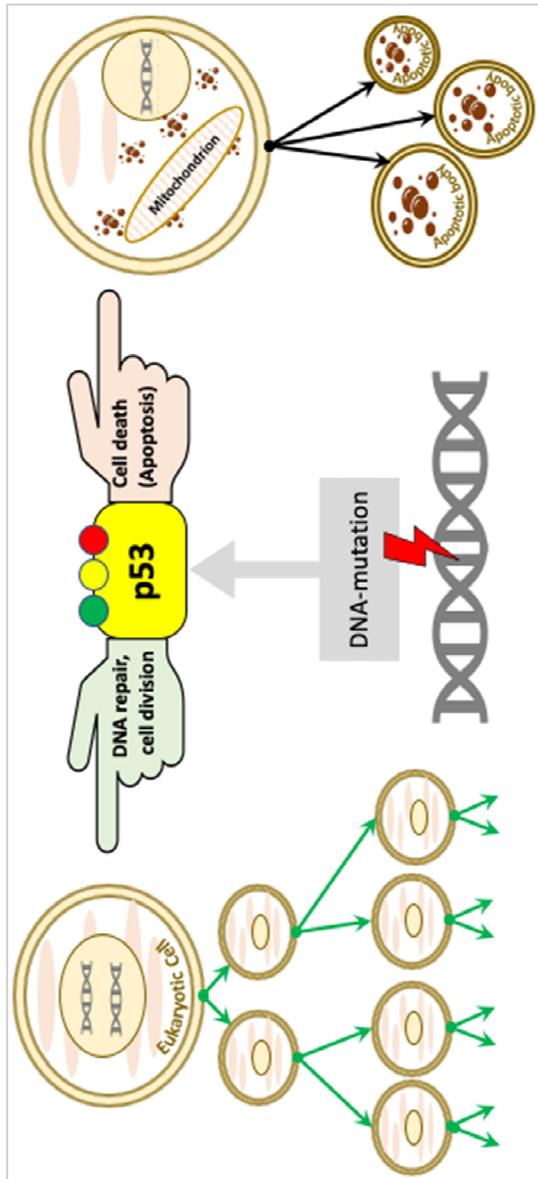


Figure 1-1: Protein p53 controls the DNA mutation state and directs the cell to repair and duplication (left) or to death (right).

Mutations in the p53 gene also occur in animals that demonstrate an unusually low incidence of cancer. However, these animals have typically found ways to overcome them. For instance, elephants keep extra copies of the “guardian of the genome” p53 gene, and therefore rarely have the disease (Callaway 2015). The cancer mortality rate for elephants is less than 5%, while for humans with only two p53 gene copies it is between 11% and 25% (Ferlay et al. 2015). Mutations in genes that encode other participants of the apoptosis pathways (for example, mitochondrion membrane proteins Bcl-2 family members or pro/caspases) may also arouse apoptosis failure at the cellular level in cancer.

1.3. The immune system controls mutations from the outside

In addition to p53, the second brake that protects the majority of us from cancer is our immune system, which works outside of cancer cells. In this disease, this external brake is damaged along with the apoptosis mechanism inside the cells. Mutations in oncogenes and tumor-suppressor genes drive the development and progression of cancer. When we think of cancer development in terms of cancer drivers, the effects of these drivers are likely moderated through the interactions of genes with regulatory functions. We must not only consider the specific tumor mutations in coding regions. Mutations in non-coding regions that serve key regulatory roles are also of functional importance. These are notoriously difficult to study and are often overlooked since they do not code for any proteins. Many mutations fall into non-coding regions and have no clear functional association with cancer drivers, but nevertheless may be important for the regulation of gene expression.

One of the major problems in cancer research is prioritizing mutations. Cancer mutations are associated not only with cancer genes, but also with many other genes related to functions that can be linked to cancer development and progression. While hundreds of drivers in coding genes are known, only a handful of non-coding drivers have been discovered to date despite an intensive search (Shuai et al. 2019). Contrast this to the fact that less than 2% of the human 3 billion DNA bases consist of protein-coding sequences. Mutations in the remaining “dark matter” of the cancer genome can drive multiple types of cancer and influence the host immune system. Little is known about cancer cell mutations that re-express genes and help them to resist the immune system.

The cancer-associated mutations can be located in the promoter regions of oncogenes and tumor-suppressor genes, but many fall outside these areas. Mutations often happen in groups of genes that regulate the immune system, which indicates the importance of immune processes in cancer. In particular, cancer-associated mutations may alter the expression of Major Histocompatibility Complex class I (MHC I) genes (which is crucial to triggering the innate and adaptive immune systems) clustered on the p-arm of chromosome 6. This indicates a potentially greater role of immune processes in cancer than previously expected (Fagny et al. 2019). Such facts necessitate the research of genes encoding and/or modulating the immune system. The progress in the field of cancer immunotherapy has renewed this urgency for basic studies on immune regulation.

1.4. Indulgence mutations

Cancer cells are more than a mass of cells growing out of control. They participate in an active battle with the immune system which protects us from invaders. The mutated cells fight for their survival; their ability to evade the immune system is a hallmark of cancer.

Mutations that affect the immune system are widespread and obligatory. I propose to call them “indulgence” mutations since they open “a way to reduce the amount of punishment one has to undergo for their sins” (Pak 2018a). Indulgence mutations are present in the majority of cancer cells and help them withstand the selection pressure of the immune system. They can be considered a fraction of “driver” mutations. Since cancer cells can be born out of mutations that occur in cancer stem cells (CSCs), representing only 0.8–1.0% of all cancer cells, most of the over-expressed proteins targeted on the cancer cell also exist in CSCs. Due to these mutations, cancer cells can avoid attacks by natural killer (NK) and T cells, and/or create a tumor microenvironment (TME) (Salvany-Celades et al. 2019).

Multiple immune evasion mechanisms help cancer grow unnoticed by both innate and adaptive immune systems. It is amazing how a cancer cell can protect itself and its progeny from the powerful immune system. Cancer can grow for months and years, while, for example, neutrophils can destroy the enemy in minutes. What type of mutations inside a cancer cell can neutralize the diverse external mechanisms that the immune system uses to erase enemies? Let us look at some of them.

Most cells express MHC I antigens on their surfaces. MHC I is an important component of adaptive immunity T and B cells. MHC I proteins, together

with foreign (virus, bacterium, or tumor) antigens on the cell surface, are presented to adaptive immunity cytotoxic lymphocytes (CTLs), triggering an immediate response against that particular foreign antigen. If the MHC I antigen presentation does not happen, the cancer cells will not be killed by CTLs.

Due to indulgence mutations, cancer cells can reduce the levels of MHC I antigens on their surfaces by various mechanisms in order to escape destruction by CTLs. For example, cancer cells can block the function of a gene called NLRC5 (related to MHC I). This enables them to evade the immune system, proliferate, and form tumors. The NLRC5 mutation rate is relatively high in melanoma patients. Expression of the NLRC5 gene is highly correlated with cancer patient survival in various cancer types, most notably in melanoma, rectal cancer, bladder cancer, cervical cancer, and head/neck cancer. The patients who survive longer tend to have a greater expression of NLRC5 (Yoshihama et al. 2016).

The importance of MHC I is further supported by the fact that some viruses (having much fewer genes than cancer cells) have to encode and rely on the same mechanism to escape elimination, i.e., they force cells to hide MHC I antigens (Bennett et al. 1999).

On the other hand, cancer cells that do not express MHC I antigens on their surfaces can be destroyed by NK cells and the macrophages of the innate immunity. Indulgence mutations enable cancer cells to secrete tumor growth factor-beta (TGF- β), which suppresses NK cells and macrophages, thereby helping the cell to survive (Batlle and Massagué 2019).

Yet another way that indulgence mutations can change cancer cells is by elevating the CD47 expression on their surfaces. This protein, which is expressed on cell surfaces in aggressive cancers, is basically the most convenient “do not eat me” signal that blocks the killing of cancer cells by macrophages. CD47 binds to a specific protein on the surface of a macrophage surface which inhibits its ability to kill cancer cells (Fruci et al. 2012).

MHC I antigens plays a central role in controlling the phagocytic function of macrophages. Cancer cells in which MHC I expression is not reduced can escape from macrophages by capitalizing on the second “do not eat me” signal on their surface: the common MHC I component β 2M that directly protects the cells from phagocytosis (Barkal et al. 2018).

Tumors are difficult to destroy because they have different defenses, including an ability to secrete proteins that systemically disable the immune system. They not only express programmed cell death ligand 1 (PD-L1) on their surface but also release lipid-encapsulated exosomes armed with these proteins. PD-L1 proteins directly bind to and inhibit PD-1 protein-expressing T cells—the base of many immunotherapies. A single tumor cell can produce numerous copies of exosomes, while the interaction between PD-L1 exosomes and CTLs provides a systemic suppression of anti-tumor immunity in the body (Dahmani and Delisle 2018).

Extracellular vesicles from human melanoma cells that upregulate PD-L1 are important in reprogramming normal myeloid cells into functional myeloid-derived suppressor cells (MDSCs). In turn, the latter suppress T cell activation (Fleming et al. 2019).

Glioma-derived exosomes contain a 25 kDa placental growth factor which is essential in inducing maternal-fetal tolerance through the innate immune response by MDSCs and tumor-associated macrophages (TAMs) recruitment. Both suppress specific CTLs and have an impact on dendritic cells (DCs) and NK cells (Albonici et al. 2019).

Summing up, the evasion strategies on cancer cells include multiple mechanisms, such as the downregulation of MHC I antigens, the secretion of immunosuppressive cytokines and exosomes, the secretion of TGF- β , the recruitment of regulatory T cells (T-regs), MDSCs or polarized M2 macrophages, or the expression of immunosuppressive molecules on the cell surface, including PD-L1 (Tuccitto et al. 2019), CD47, and others.

There exists, however, an even more common and powerful mechanism to avoid execution by both adaptive and innate immunity.

1.5. Re-expressions of oncofetal genes as indulgence mutations

The same normal (non-mutated) genes that prescribe how fetal cells multiply, migrate, and create a newborn child are among those that are necessarily present in cancer. They can be awakened by mutations in non-coding regions and cause a tumor. Oncofetal genes are expressed in developing as well as in cancer cells. This expression serves the functions that are essential during healthy development and which are also re-activated during cancer. Sometimes these functions involve different gene promoters or transcription alteration; they may participate in processes, such

as pregnancy, inflammation, and antibody overproduction, where immune reactions are modulated or canceled.

Cancer cells accumulate mutations that exploit normal immune reactions “at the wrong time and in the wrong place”. Indulgence mutations allow cancer cells to upregulate the expression of genes that are involved in immune tolerance during pregnancy.

For instance, several cancers re-express embryo secreted alpha-fetoprotein (AFP). AFP is the first α -globulin to appear in mammalian sera during development, and the dominant serum protein in early embryo life.

AFP did selectively induce a rapid downregulation of surface MHC class II antigens in their expression on human monocytes, thereby making embryo/tumor cells “invisible” to the immune system. MHC class II antigens on monocytes are the key molecules in antigen presentation. They serve to differentiate between a self-cell and “alien” embryo and cancer cells. AFP neither alters the expression of MHC I, CD4, CD18, CD45, and Fc receptors for IgG on the surface of monocytes/macrophages nor affects the functional maturation of the macrophages Fc receptors or the ability to express antibody-dependent cell-mediated cytolytic activity (Laan-Pütsep et al. 1991). AFP may, by reducing the antigen-presenting capacity of monocytes/macrophages, function as an essential factor in maintaining a fetal allograft, as well as participate in the downregulation of the entire immune system in cancer.

AFP reappears in the adult serum during individual pathologic states, primarily in hepatocellular carcinoma (HCC). AFP attracts immune-suppressive MDSCs and T-regs through a specific AFP-binding receptor: C-C chemokine receptor type 5 (CCR5) (Atomezem et al. 2002; Umansky et al. 2017). As a result, MDSCs and T-regs migrate and accumulate, and thus suppress the immune attack on cancer.

The majority of cancers re-express another AFP-binding receptor (AFPR) (Laderoute 1991). AFP’s primary function is the delivery of nutrients to cells via AFPR-mediated endocytosis. AFP delivers nutrients to embryo/cancer cells and stimulates their growth.

Mutations are random, and AFP and AFPR can be re-expressed by cancer cells independently. AFPR re-expression is essential and critical for the survival of cancer cells, while AFP can be secreted by the host during an on-going hemopoiesis, injury, regeneration, or inflammation. Furthermore,

inflammation is directly linked to cancer (Ostrand-Rosenberg and Sinha 2009).

Mutations that enable AFPR and AFP re-expression can be considered to be indulgence mutations in cancer cells. The AFP-AFPR nutrient delivery duo serves embryo cells and MDSCs during pregnancy, and cancer cells and MDSCs during cancer (Fig. 1-2). Cancer cells and MDSCs share AFP and in this way generate a protection shield from both innate and adaptive immune attacks. Indulgence mutations help cancer cells withstand not only the innate immunity which they have to deal with first, but also the adaptive immunity, which requires a large number of growing cells or antigens.

A lot of effort has been devoted to understanding the cause of the disease, including mutations. However, our goal is to find the most effective targets for anti-cancer gene- and immunotherapy and kill cancer cells, whatever mutations they may have accumulated. Indulgence mutations that trigger the immune system suppression can be found in the dark matter of cancer genomes. They can be possibly fixed with new genetic engineering technologies using, for example, a modified prokaryotic immune system CRISPR that confers resistance to foreign genetic elements. Alternatively, solutions for the cancer problem may also be found on the protein level and not the genes.

The most rational way to treat cancer is to fix the existing cancer brakes that perfectly protect the majority of us during our lifetimes. Dysfunctional apoptosis at the cellular level and AFPR re-expression at the body level are both obligatory for cancer cells to survive.

To restore the apoptosis inside cancer cells, we cannot adopt the solution found by elephants, but must develop other approaches. In particular, cancer cells with mutations in the genes of apoptosis pathways can be targeted by chemotherapy that employs undamaged apoptosis participants (which will be discussed later).

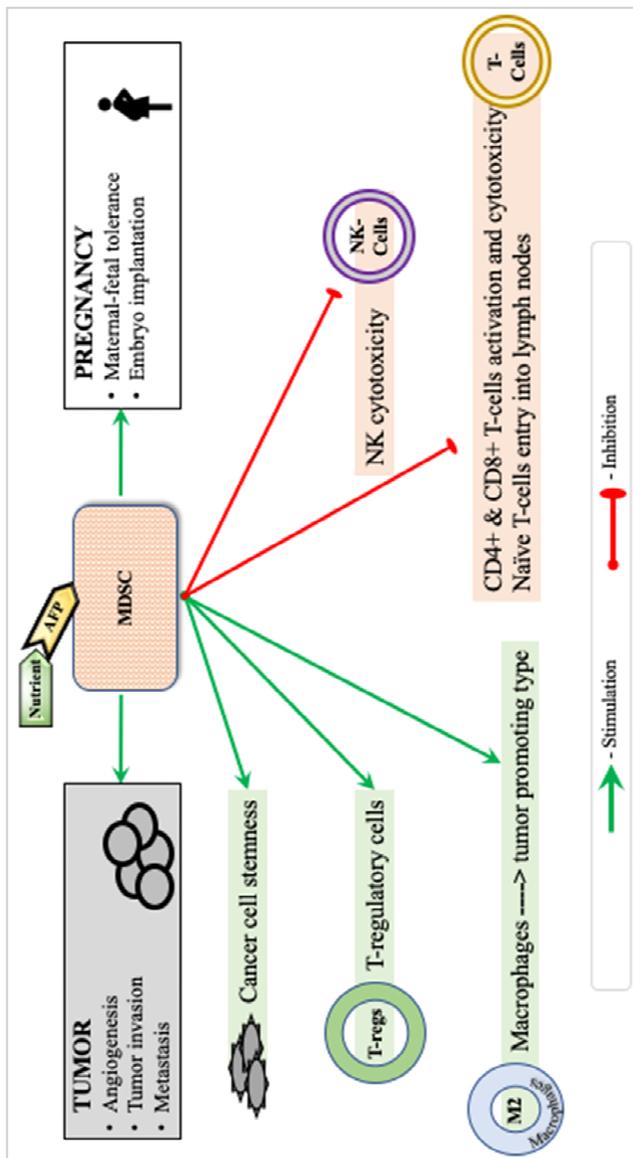


Figure 1-2: The beneficial impact of MDSCs on pregnancy and tumor tolerance. Adapted from (Ostrand-Rosenberg and Fenselau 2018).