im very honored to introduce todays

goddessman has been deputy director for

the intramural research at the nih since

99

he is a graduate of harvard college and

harvard medical school

dr gottisman completed his residency at

brigham hospital in boston

he was a research associate at the nih

from 9 to

he then returned to harvard medical

school as assistant professor before

returning to the nih in 9

dr gottisman

became the chief of the laboratory of

cellular biology in the national cancer

institute in 990

during his years of service

in the us public health service as a

commissioned officer he achieved the

rank of twostar rear admiral as

assistant surgeon general his research

interest has mainly focused on how

cancer eludes chemotherapy

hes published extensively on this

subject with over 00 scientific

publications

dr gottisman is elected fellow of aas

and the american association of

physicians and a member of the national

academy of medicine and the american

academy of arts and science im sure you

will enjoy todays lecture

my name is michael godisman im the

chief of the laboratory of cell biology

at the center for cancer research at nci

at nih

and my talk today is about the structure

and function

of abc transporters in health and

disease

this work began more than 0 years ago

as an effort to understand why it was

that some

cancer cells were resistant to

anticancer drugs

whereas others responded well to

chemotherapy

and led as you will see to the discovery

of

transporters that are involved not only

in drug resistance in cancer

but in

handling drugs many drugs in different

compartments

in the body as well

so um

our overall goals when we began this

work in in current studies as well were

to define the molecular mechanisms of

drug resistance and cancer

and we focused mostly on natural

products these are drugs that are

traditionally used to treat cancer and

to which many cancers respond quite

effectively and also compounds which

include platinum such as cis platinum

which are also effective anticancer

drugs

we discovered in the course of our

studies a number of different mechanisms

of resistance to these agents

and weve been engaged in trying to

determine the clinical relevance of

mechanisms derived from these in vitro

studies to actually affect resistance in

patients tumors

this work has helped us develop new approaches to exploit or circumvent clinically significant resistance mechanisms and has led to a better understanding about the cellular pharmacology and the formal kinetics of drugs in the human body

now the issue of drug resistance and cancer can be summarized in this slide and that is for most metastatic cancers those are cancers that have spread from one part of the body to another chemotherapy with classical or targeted anticancer drugs can result in remissions but frequently does not cure the cancer

and this is shown schematically here as a population of cells that may be intrinsically resistant and so not responsive to anticancer drugs at all or essentially sensitive but containing some resistant drugs which over the course of therapy

become the major population within the tumor and so the tumor acquires

resistance

there are two major hypothesis

hypotheses to explain the development of

multidrug resistance

which can result in treatment failure

the first is that within the initial

population of the tumor of tumor cells

there are a few cells perhaps a small

subpopulation that are already resistant

to the therapy which is going to be

employed

evidence exists in acute myelogenous

leukemia and in melanoma that that is

indeed the case and that

when you treat a tumor you get

remissions and the cells that grow back

were originally present in the original

tumor and that can be demonstrated using

sophisticated molecular techniques

the second basic model is that there may

be resistant and sensitive cells in the

initial population but that during the

course of therapy additional mechanisms

of resistance develop

and that these may become fixed during

the course of

therapy leading to

mixed resistant populations

although these seem to be complementary

mechanisms the approach to dealing with

this kind of resistance is quite

different

in the first case you want to derive a

therapy which kills as many of the

different kinds of resistant cells as

possible in the first treatment

whereas in the second case its really

not known what kinds of mechanisms of

resistance will derive and therapy needs

to be personalized for each tumor so

that you can identify

which mechanisms may be present in an

individual patients tumor

this of course makes therapy and much

more difficult

and in many tumors this appears to be a

major way in which resistance develops

our goal was to understand at the

molecular level what mechanisms might

pertain in each of these cases

so

let me take a step back before i go into

some details and describe

some of the specific mechanisms that

occur that cause resistance in in tumor

cells

for

therapies that are targeted that are

aimed at specific oncogenes that may be

responsible for tumors

we often see mutations in these targets

or bypass mutations for example if the

target is a

growth factor receptor

and the

the drug no longer inhibits that

that specific target

you may see alternative ways of

activating

the tumor using other kinds of pathways

other than the pathway that is being

targeted

a second mechanism shown to the right

here

is that altered uh cells may show

altered cellular pharmacology and this

is a mechanism weve been studying in

considerable detail and has enabled us

to develop an understanding of how drugs

are handled in the body

based on how cancer cells handle these

drugs and this includes mechanisms that

may reduce the accumulation of drugs or

alter metabolism of drugs or increase

influx of drugs from cells

a third change which turns out to be

rather important in development of drug

resistance is changes in differentiation

pathways

and homeostatic responses within tumors

so a tumor that begins as a epithelial

kind of tumor can change into a more

mesenchymal tumor with uh with massive

changes in gene expression resulting in

very different patterns of resistance to

anticancer drugs and the classic

example is whats called epithelial to

mesenchymal transition

and finally there are important uh

alterations in the local environment of

tumor cells

including the presence of other cell

types that are not cancers but can

affect response to chemotherapy

changes in the

in the substrate in which the cells are

sitting including changes in tissue

plasticity and changes in elasticity

and alterations and mechanisms including

immune mechanisms and mechanisms that

affect

growth of blood vessels into tumors all

of these undoubtedly contribute to drug

resistance but our effort has been

focused on those that change cellular

pharmacology

now if you look at cellbased mechanisms

of resistance there are three general

classes of

altered alterations in the tumor that

can result in reduced

reduced efficacy of chemotherapy

the first and most obvious is that the

drugs simply dont get into the cells

we know there are

close to

00 different

solute carriers which are responsible

for moving

normal nutrients and other agents within

cells

and approximately 0 of them have been

demonstrated to also affect uptake of

specific uh drugs into cells and and

reduction in amount or changes in the

specificity of those transporters can

produce drug resistant cells

if the if the cell if the drugs can get

into the cells there are mechanisms that

affect cell biology

which have profound effects on drug

resistance such as reduced

cell killing by cell killing pathways

known as apoptosis

altered cell cycle checkpoints or growth

pathways

altered metabolism of drugs within the

cells

altered targets or increased repair of

damage and even compartmentalization of

drugs within subcellular compartments

that prevent them from reaching their

targets

now much to the surprise i think of many

people in this field

it was discovered approximately

now 0 now to 0 years ago that a

major mechanism of resistance is

actually related to the increased energy

dependent efflux of drugs from cells

and we were involved in cloning some of

the original transporters that were

responsible for efflux of drugs

and it was soon discovered that these

belong to a family of atp dependent

transporters called abc transporters of

which are known in the human

now

these transporters turned out to be

particularly interesting both in terms

of their biology

their biophysics

and in their cellular pharmacology

weve shown that they play an important

role in multidrug resistance in cancer

and this is true also in

normal cellular pathogens

including

protozoans and bacteria

these kind of transport systems turn out

to be rather important

they play a role in pharmacokinetics

that is the uptake distribution and

excretion of drugs in the body

they are important in drug toxicity

because

tissues that have efflux pumps are less

likely to be affected by drug toxicity

one example that ill mention for

example is the stem cells in the um in

the blood in which case expression of

these transporters makes stem cells

somewhat more resistant to anticancer

drugs

than you would expect otherwise

they play a key role in development

i mentioned their expression in stem

cells theyre involved in morphogenesis

because they can keep

normal products from entering cells

and weve learned that they are

important in understanding the biology

of all transport systems

so uh let me focus now on the atp

binding cassette or abc transporter

superfamily

it is one of the largest families of

transport proteins known currently more

than 000 members have been identified

substrates include many different kinds

of small molecules ions sugars glycans

phospholipids cholesterol peptides

proteins toxins

antibiotics

and as ill soon show you hydrophobic

natural product anticancer drugs

structurally they consist of various

combinations of atp binding domains and

segments that include transmembrane

domains

the family

of eukaryotic abc transporters

includes different known genes

and this shows the evolutionary profile

of these different genes mike dean

a senior investigator at nci

has really elucidated these various

families

and you can see that the human

transporters are closely related to

those in primates

but also to chicken and other mammals

and reptiles as well

every single known

family of

living organisms includes

representatives from this family

now uh in the human as i mentioned there

are human abc genes they fall into

seven different families

and the families are defined by their

sequence homology but also by their

structural homology so

for example

in the abc a family there are members

and all of them as shown at the bottom

right

consist of

two segments each of which contain six

trans membrane domains

and two atp binding cassettes

for the abcb family

which includes some several members

of the family that includes multidrug

resistance proteins

you can see that again we have six

transmembrane domains

and two atp binding cassettes but there

are members of this family as for the

abcg family that include only a single

segment that has transmembrane domains

and one atp binding cassette and

evidence suggests that these form dimers

so that the coin of the realm here are

the two

six trans membrane domain segments and

the two atp binding cassettes

now the transporters are involved in

many different

physiological processes

there are three that seem to have a very

wide specificity are multidrug

transporters

and those are known as abc b

generally called pglycoprotein or pgp

this was the first of the family to be

discovered in the 90s cloned in the

90s and led to the discovery of the

other transporters

abc c or mrp multidrug resistance

related protein

is also expressed in many different

tissues as is abc g

also known as bcrp standing for breast

cancer resistance protein

and mitosanthrone resistance protein and

many different laboratories have been

involved in identifying cloning these

transporters and understanding uh their

drug specificity

if you look to the right you can see a

list of

different drugs that are

[Music]

substrates for pglycoprotein

since pglycoprotein sits in the plasma

membrane

these drugs are unable to accumulate in

cells that express high levels of

pglycoprotein

and the tissues that express this

protein are shown in the lower left

column and i will describe in a moment

in a little more detail

how these affect the distribution of

drugs within the body

now

its been demonstrated that there are a

number of different human diseases

where expression of transporters affects

the the development of the disease and

in most cases these are simple mendelian

dominant disorders

i mentioned cancer in the multidrug

resistance genes but for example cystic

fibrosis the gene that is responsible

for this very common genetic disorder

which leads to lung disease in human

populations

is the transporter abc c

in this case the primary function of

this transport system

is as a chloride channel but its a

regulated chloride channel

and the abc binding cassette is involved

in opening and closing of the channel

and then you can see from this list that

there are a variety of other diseases

and although we do not know diseases

associated with eat with all of the

transporters uh this list continues to

grow and it looks as if

most of them subserve specific functions

when they are absent the effect is not

lethal but it does result in disease

which can be quite debilitating for for

human beings who have these problems

so to get back to those three multidrug

transporters abc b abc c and abc g

their overall structure is shown here

as you can see as i mentioned for abc b

there are two regions which have six

transmembrane

domains and there are two atp binding

cassettes

for abc c theres a an extension at the

end terminus of five transmembrane

domains

evidence exists that that can be deleted

and the and the abcc one is still a

multidrug transporter and its thought

to be involved perhaps in localization

of the protein or stabilization of the

protein in the plasma membrane

as i mentioned abc g another multidrug

transporter which shows only one segment

of transmembrane domains

appears to exist in most cases as a

dimer

fulfilling the requirement that you see

for abc b

now

in the human these three multidrug

transporters have some overlapping drug

specificity

this is shown in this venn diagram if

you look in the middle

you can see a variety of different

anticancer drugs including both

targeted anticancer drugs and more more

more classical drugs that are

not specifically toxic but do kill

cancer cells

these are very effective anticancer

drugs but in the presence of any one of

these transporters

uh their efficacy is reduced you can

also see that there are specific agents

including anticancer drugs and other

intermediate metabolites

that are affected by one or the other of

these specific transporters

now this is the situation in the human

most of our studies on understanding the

pharmacology of drugs and the effect of

these transporters is done in rodent

systems either the mouse or the rat

and in those cases the specificity of

each of these transporters is slightly

different

so being able to extrapolate from what

we learned in the rodent systems to

human is not necessarily totally

straightforward

in many cases the specificity is similar

but in some cases it actually differs

so

let me get back to this issue of what

the normal function of pglycoprotein is

and the information is provided mostly

in localization studies

it sits for example in the small and

large intestine

on the luminal surface

so that if drugs are taken up orally the

ones that are substrate for pgp

are unable to be absorbed because as

soon as they enter the plasma membrane

of the intestinal epithelial cells

theyre pumped back into the lumen and

they are fecally excreted

the transporter is also present at high

levels in the biliary epithelium

and so some of these drugs are

excreted into the bile its in proximal

tubule cells of the kidney so drugs can

appear in the urine

and it plays a really important role at

the bloodbrain barrier and in other

barrier sites

such as the blood testis the blood ovary

and the blood placental barrier

pumping material out of these

specific organs to protect them

and

it also can be expressed in either tumor

cells or circulating cells

in the vascular space or the

interstitial space conferring resistance

to anticancer drugs and insensitivity

to other pharmacological agents for

which

the p glycoprotein is a transporter

so in our early studies we asked what

the relative specificity was of these

different transporters for conferring

resistance

we did these experiments in a couple of

ways one was to simply measure the level

of transporter in different cells

and find out what

transporters were responsible for

resistance to what agents

the other way was to select for

resistance of specific

to specific drugs

and ask where where over expression of

transporters conferred resistance and

you can see

that almost all of the standard

anticancer drugs

can we can find at least one transporter

thats responsible for creating

resistance either by selection or simply

by measuring expression of uh high

levels of a transporter that confer

resistance to these drugs

so um its its its almost a truism

that no matter what the agent thats

used to treat cancer

one or more of these transport systems

will effectively pump drugs out of the

cells

that doesnt mean that all anticancer

drugs express these transporters but

when they do

its pretty clear that resistance will

develop

so id like to focus a little more now

on the biophysics and physiology and

biochemistry of these transport systems

this is a linear diagram showing the

0 amino acids that make up human

pglycoprotein

this protein consists of two halves and

a connecting region that connects these

two halves

as you can see there are phosphorylation

sites um in the region that connects the

two halves of the protein these are

shown in red

weve deleted those sites and made made

it impossible to phosphorylate them

and it doesnt look as if theres much

effect on the transport activity we

cant rule out some regulatory effects

of phosphorylation but phosphorylation

is not essential for function

the yellow circles represent the regions

uh the the parts of p glycoprotein in

which mutations have been shown to

change drug specificity

uh these are clustered around the

transmembrane regions

and as ill show you in a moment this

led to the hypothesis that the

um the place in the transporter in which

the drugs interact is actually within

the lipid bilayer

and thats uh also supported by photo

affinity labeling studies

which are shown in green here in which

transmembrane segments to and

9 to are specifically photo affinity

labeled

consistent with the data from the

genetic studies showing that these are

important regions for drug binding

within the transporter

this led to a model that we proposed in

about

9

which specified that the transporter

sat in the plasma membrane that the site

of recognition of the substrates was

within the plasma membrane and and i

just need to let you know that virtually

all of the substrates for these um

anticancer transporters anticancer

drug transporters are very hydrophobic

and in biophysical studies they prefer

to partition into lipid bilayers

compared to cytoplasm or extracellular

space

and this model was proposed quite as i

mentioned quite some time ago

suggesting that the transporters were

recognizing drugs within the lipid

bilayer and that we did know at that

point that both atp sites were essential

for function

and we were interested in understanding

the mechanism by which

many different drugs could be recognized

and the binding of the drug led to

activation of atpas and the eflux of

drug from the cells

several years ago it became possible to

get crystal graphic structures of

pglycoprotein and other aebc

transporters

and

steve aller and jeff chang published now

several years ago the structure of the

mouse p glycoprotein thats shown in the

diagram on the left

this was not an extremely high

resolution structure

but it demonstrated

that a substrate could actually bind to

p glycoprotein and indeed

was binding within the transmembrane

regions in the lipid bilayer

in the structure that was published the

two atp sites were very far apart

and evidence suggested that the active

form of the protein in which atp could

be hydrolyzed the atp sites were close

together thats shown in a model on the

right

which kasper local published

looking at an abc transporter from

from a bacterial species staff aureus

the model that we have is that um the ap

glycoprotein exists in both states

and that when drug is bound

the two sites get together and when they

get together

atp is hydrolyzed and drug can be

extruded

now what is the is the

is the evidence for this

so

my colleagues in the laboratory of cell

biology suresh umbudkar

and sriram subramanyam

were able to develop cryo

with sunni shuklas

support were able to develop cryoem

structures this is a technique for

using a very high resolution electron

microscopy to study structures of

different proteins

uh and as you you can show and see in

the upper left

we found roughly equal amounts of the

open and the closed form of pgp

and

based on the fact that in studies in

which

atp hydrolysis could be blocked with

vanadate

and we found only the closed form and

therefore the active atpase form of pgp

weve postulated that theres a cycle

in which the open and the closed form

are

intermediates a substrate can bind to

either of these forms

when atp binds the only form thats

present is the form in which the two atp

sites are close together once atp is

hydrolyzed the

the p glycoprotein changes its structure

releases the drug into the extracellular

space

and then is free to recycle again

now the precise way in which substrates

were recognized by the transporter was

also quite mysterious

and disha in our laboratory was able to

generate roughly nine different crystal

graphic structures of pgp

and he found that the two atp sites were

variable distances apart

in this series ranging from angstroms

to 0 angstroms

and that when he looked in detail at the

transmembrane regions the parts of the

transmembrane regions that were exposed

in the with the different distances

between the atp sites were different

so that as the two atp sites moved

together or apart as they did under

equilibrium conditions

different residues were exposed in the

transmembrane regions allowing the

binding of many many different

substrates

so this led to the following hypothesis

the motion of the two nucleotide binding

domains with respect to each other

creates torque in the transmembrane

domains that bind substrate

this allows pgp to sample a large number

of potential substrate interaction sites

in the transmembrane domains and this to

some extent may account for the enormous

lack of specificity or at least the

specificity for many different drugs

that are recognized by this transporter

the proof that this is actually the

model

will require much higher resolution

crystallographic structures with

different

substrates bound to demonstrate that

different substrates bind to different

preglycoproteins in which the atp sites

are at different distances from each

other

and that as we change the distance

between the two atp sites we change the

substrate specificity of the transporter

and these studies are in progress

so um id like to end by uh getting back

to this issue of what the role of

pglacier protein is in cancer

we believe that approximately 0 percent

of human cancers express pglycoprotein

at levels sufficient to confirm

multidrug resistance

and this has been shown in multiple

studies

pglycoprotein is commonly expressed in

a lot of common cancers

for example

in tumors that have been selected for

resistance to drugs we find pgp

expressed at high levels in leukemias

myeloma lymphomas breast and ovarian

cancer

and that in situations in which we

inhibit the expression of pgp

the sensitivity of these tumors to drugs

can be reduced

we also see p glycoprotein in cancers

which express pgp at the time of

diagnosis

including many solid tumors such as

colon cancer kidney pancreas and liver

cancer

and its generally known that these

cancers dont respond to pgp inhibitors

up front so they are intrinsically

resistant and the tumors i mentioned

before are

demonstrate acquired resistance

in animal models using human cancer

xenografts or models in which tumors are

derived directly from

the animals themselves

show that expression of pgp is a major

mechanism of resistance that inhibitors

of pgp confer

sensitivity

and this has led to

an optimistic view that much of drug

resistance might be reversible by pgp

inhibitors

however

progress so far has been slow

although a number of different pgp

inhibitors have been tested in many

cases only transient response or no

response at all

has occurred

suggesting very strongly that although

pgp may be

sufficient for

drug resistance its not necessary

it may coexist in many cases with other

mechanisms of resistance

and so our search for

all the different mechanisms that confer

resistance goes on

but in the meantime weve learned quite

a lot about how drugs are handled in the

body

in another lecture in the series matt

hall will talk specifically

about the role of pgp

abc g and abc c and the blood brain

barrier and the blood placental barrier

and i think youll see at that point how

the studies that ive described today

are leading to a much better

understanding of drug drug interactions

and the normal handling of drugs within

the human body

so thank you for your attention

and on at this point i will end my

lecture