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Interview by Ute Deichmann with Howard (Chaim) Cedar, Hebrew University of Jerusalem, School of Medicine

Followup Interview- 4 August 2014

DNA Methylation and Cancer

UD: To connect where we ended last time, when you talked about the booming of the term "epigenetics". You said that many researchers use the term when they don't know the cause of a heritable phenomenon, which is probably often not justified.

Of course, there are diseases and other phenomena which can be related to DNA methylation. From what I have read on your home page, you are now working on the role of DNA methylation in the origin and treatment of cancer, is that correct?

HC: A little bit. We do work on that.

UD: I would like to know if methylation is the cause or the result of the changed behavior of DNA in cancer cells?

HC: This is a very good question. We don't know the answer. It's a logical issue. We know that in every tumor that everybody looked at, there is abnormal methylation. If you look at the methylation pattern of the normal cell and you look at the methylation pattern of the cancer cell, there are a lot of differences. One of the major differences is that there are sites in the normal cell that are not methylated that become methylated in the cancer cell. And there have been a number of studies that have shown that if you take cancer cells and grow them in tissue culture and you treat them with a drug, 5aza cytidine, which lowers the amount of methylation in the

cell, the cells lose their tumor properties. They go back to being more normal.

UD: That's surprising, given that methylation inhibits gene expression

HC: I'll give an example of how it can be explained. Most of the genes that are involved in cancer are called tumor suppressors. Their job is to prevent tumors. We have a lot of these genes – a very large number. It makes sense that man and other mammals should have genes to protect them from becoming cancerous. In cancer, you find a lot of these genes have become abnormally methylated - which means that they don't function any more, which means that they're not protecting the cell from cancer. So you're right – it's counter-intuitive, but it works something like that.

If you take cells in culture and you treat them with this drug, they lose many of their cancer properties and start to grow like normal cells. This has been used as proof that methylation causes, or is involved, in cancer.

So the question is, how does this happen? What is the role of methylation in a person or a mouse? The dogma has been the following: you have a normal cell which is transformed by a mutation, or a virus, or by some sort of trauma, or by inflammation – something that disrupts the DNA of the cell and causes its destruction. That starts the tumor process. Then, the dogma says, one of the things that happens as a result of that, is that there is abnormal methylation. And that just makes the situation worse.

There's no proof of that idea.

UD: But you said it's already a dogma.

HC: It's a dogma, but it's a dogma that might not be true. I'll tell you one of the reasons why I think the model is not correct. If you take the normal cell and you take the cancer cell and you say this gene is unmethylated here, and is methylated here, the usual assumption is that here it's active and here it's inactive because it underwent

methylation. That's not the case. If you look at the genes that get methylated, they're already inactive.

UD: That's what you told me last time.

HC: They're already inactive. So the methylation is not making these genes inactive.

So what do I think is the model? The model is that during life – during aging – there is a natural process whereby many sites in the genome get methylated abnormally. It's a slow process – it takes 90 years. And slowly the amount of methylation goes up in all tissues. That is true. There is no question about it.

When I was a kid we used to go to the local fair in town. In the summer. There was always a man there that would take your money and tell you that he could determine what age you are. And you used to give him a dollar, and he would look at you and touch you and pick you up, and he would say, "you're 34 years old." And most of the time he was close.

I always joke that I can do it more accurately than he can. You just need a little bit of blood, I test the amount of methylation, and I can tell you somebody's age within a couple of months. It's very accurate. Methylation is increasing all the time. It's not uniform – it occurs in some cells, and in others it occurs less. It's random, but some cells are more prone.

I think that that makes cells more prone to cancer. If a mutation occurs in a cell that hasn't been highly methylated, the mutation won't do anything. But if the mutation occurs in a cell that, through aging, has gotten considerably methylated, then that mutation can make the cell cancerous. So I think methylation plays a very important role, but it's not an outside influence – it's something that happens naturally.

There's a lot of evidence for this concept. All of these genes that get methylated are involved in development and differentiation. And usually a gene like that – let's say it's involved in differentiation of

colon tissue - is inactive in all cells of the body. It's not methylated – it's inactive because there are other mechanisms that make it inactive. Proteins bind to it and make it inactive. Then, when the cell wants make the colon – or use the cells for colon, it gets rid of the repression and turns on the gene. And that helps the tissue differentiate. My claim is that if, early on, this same gene is getting methylated through aging, when it comes time for this cell to differentiate, it can get rid of the repressor – that's easy – but it can't get rid of the methylation. And the methylation doesn't allow it to differentiate. And cells that can't differentiate are prone to cancer, because cells always divide until they differentiate. Differentiation saves them from division. So because they are methylated, they can't get turned on, and can't get differentiation, and are now stuck in a state in which they want to proliferate. And that's conducive to cancer. It's not enough for cancer, but together with a mutation, or two mutations, that brings on cancer. So I think that's the way it works.

UD: Together with a mutation, or caused by a mutation?

HC: Independently, there's a mutation.

UD: How come the repair mechanisms are going down with age?

HC: One of the reasons the repair mechanisms go down is because of this methylation. The repair genes are supposed to be unmethylated. Those are genes that undergo methylation with time. The repair ability of the cells is damaged by aging.

Did I tell you last time about colon cancer and the mouse? There is a mouse that has a mutation from birth – scientists gave it to him – and it makes him prone to colon cancer. All these mice, when they get to the age of 4 or 5 months, they develop many, many tumors in their intestines. There is a similar mutation in man that does that. This is similar to the breast cancer gene. Women have this mutation to begin with and they develop breast cancer at some age. The same thing is in our mouse.

So somebody took this mouse, about 20 years ago, and treated it from birth with low doses of 5-aza-cytidine. That's a drug that prevents methylation. So the animal is born, and once a week he is injected with a low dose of this drug for his whole life. Without treatment, these animals develop about 100 tumors in their colons. With this treatment from birth, they develop 0 tumors. In other words, by preventing the methylation of aging the tumor was prevented. You can show, for instance, that if you start giving the drug not at birth, but at three months, there's no effect. It all has to do with preventing the methylation of aging.

And here's a case – like in man – where there's a mutation that makes these animals prone. 100% of the animals get 100 tumors, but by preventing the methylation, they don't, even though they had the mutation.

UD: That means that the cause is basically in the mutation but you can treat it on the level of the methylation.

HC: If you look at the big picture, you need mutations to get the tumor. But you also need the methylation; without the methylation you don't get the tumor.

It could be, also, that this methylation is affected by other things. We don't know. It could be affected by inflammation, it could be affected by trauma, it could be affected by a virus – who knows?

So that's another way of looking at methylation and cancer. And I think that more and more people are starting to look at it that way, but not everybody agrees to that.

UD: For scientific reasons, or rather because of their philosophy?

HC: The model, and if you get down to it, the philosophy of the model is vague. I claim that most people have the wrong conception – that methylation is something that can inhibit expression. And that's wrong. Methylation can prevent activation; it doesn't actively inhibit. It can prevent the activation of genes in a stable manner. My model of

methylation and cancer is consistent with that. It says that methylation doesn't come and turn off genes. Methylation prevents them from being turned on.

So philosophically this model fits nicely with what methylation does.

UD: If you predict the importance of this research for the future, does it lie mostly in the treatment of cancer?

HC: It could. This drug, 5-aza-cytidine, is a drug that's in use. It's used for treating a number of different tumors. Up until about five years ago the drug was being used inappropriately by doctors. They were using it in high doses, and in high doses this drug kills cells. So it was working like chemotherapy. And then about five – maybe a little longer – years ago, they realized that if you use a lower concentration of the drug it doesn't kill cells, but inhibits methylation. And now there's been a bit of a renaissance in the use of this drug. They tried all over the place with a lot of successes. Most of this has not been published. But the place where it works the best is in a disease called MDS – Myelo Dystrophic Syndrome. It's a form of leukemia. It works the best there because it is leukemia where it is known that the cells can't differentiate. That's the main cause of this leukemia. You treat it with this drug and the people get better and don't go on to the next stage of the disease.

So it's an up-and-coming thing and I don't know where it's going to go. I would like to see it go to a place like the mouse experiment. I would like to see it go to a place where when children are born we give them something and it will inhibit the methylation of aging and they'll be healthier. We're obviously very, very far from that. But it has potential.

DNA methylation and trans-generational inheritance

UD: I'd like to return to what we discussed last time regarding early mammalian development. Gary Felsenfeld told me that, in general,

DNA methylation lasts for three or four generations and then it gradually dissipates because the patterns are not faithfully copied and there is no error correction during replication. He referred to the transmission via the germ line, not via mitosis. How faithful is the copying of the methylation pattern?

HC: This is an interesting subject. Gary might have been referring to the tissue culture, where methylation is not necessarily maintained. But I'll tell you what goes on during early development. Here the methylation patterns in the gametes - the sperm and the egg - get erased. So in the very early embryo, there is very little methylation and erasure. But then the methylation pattern is established by rules. According to that concept, if you ask whether a methylation change during the lifetime of a person can be passed on to children, the answer is no.

Why is that? Let's say that when I was 20 years old I had a change in methylation in some of my cells. Of course, it's irrelevant because if it's not in the sperm it won't get passed on to my children. So let's talk about an experience in life such as starvation conditions for a year in a concentration camp. That affected methylation in sperm. When the sperm is passed on to the children there will be a different methylation pattern. That will affect the child. But according to what I said, everything is erased. I think that that's more or less true. The "out" for that is that there are sequences in the genome whose methylation pattern is not erased. Those are the genes that are imprinted. There are genes where one allele is methylated and the other allele is not. And that's not erased because the early embryo has a way of recognizing those genes and not erasing them.

So if there is a mechanism to recognize other places in the genome - not imprinted genes, but other places - and as a result not erase them, that kind of site can be inherited. So in principle it is possible.

UD: Is it very rare?

HC: I think so. Trans-generational inheritance has been shown in a number of different organisms. One organism where it has been shown to be very common is in plants. Plants don't erase their methylation pattern. So if the gametes of the plants get methylated or de-methylated during the lifetime of the plant, this will be passed on to the offspring. It happens all the time, and it's an important mechanism that plants use to protect themselves against the environment.

Another organism where trans-generational inheritance has been shown is in the worm C. elegans. C. elegans doesn't have DNA methylation. There the mechanism is different; it has to do with RNA. There's a beautiful paper now by an Israeli – Oded Rechavi – in Tel Aviv. He just published in Cell and showed that if you expose worms to starvation conditions they make this RNA – they amplify this RNA in the germ cells, and that gets passed onto the next generation. It affects the next generation's eating behavior. And that lasts for three or four generations. Then the RNA that it made gets diluted out and there's no more effect.

UD: Is this biologically sensible?

HC: It could be. You could think of it as a shortcut to evolution. It's much easier to change methylation than it is to make a mutation. So if you're making changes in methylation that could be carried on, the organism can evolve faster.

UD: But in stable environment it would not be very healthy, right?

HC: But mutations are also not very healthy.

DNA methylation and Development (2)

UD: Last time, you said that at the time of implantation almost the whole genome, except for the CpG Islands, gets almost indiscriminately de-novo methylated and that pattern stays for the whole life. But if that

pattern stays, then how is it possible that different layers and tissues are created?

HC: Again, islands are protected. When I spoke before in cancer about genes that get methylated, those are the ones – the islands that were originally unmethylated and they are supposed to be unmethylated. And a certain fraction of them can get methylated during ageing.

So at the time of implantation the whole genome gets methylated (except for the islands). During the subsequent process of differentiation there are changes in methylation taking place. New methylation – de novo methylation – on genes that were unmethylated islands.

UD: So these changes are only on the islands, because the rest stays methylated?

HC: The islands are the ones that are unmethylated. As cells differentiate, some genes undergo demethylation and other places undergo de novo methylation. So in each tissue you see its own methylation pattern.
How does this occur? Again, you have to think of methylation in keeping with the idea of it being used for stabilization – stability. It's not a cause of turning on a gene or turning off a gene. So let's say we start off with the embryo that's now gotten methylated and now it's going to differentiate into germ layers – ectoderm, endoderm, etc. Then the mesoderm is going to develop into muscle and fibroblasts and cartilage. The ectoderm is going to develop into skin and brain and neurons, and the endoderm is going to develop into all the internal organs – the liver and the colon and the lungs. At every stage there are changes in methylation. Most of those changes are secondary. There are transcription factors, master genes that cause the differentiation. Then they get stabilized by the change in methylation.

If I look at a fully differentiated cell and I see that there are places in

the liver where there is demethylation, it's only a tiny, tiny fraction of the genome. Everything else is methylated.

UD: This is surprising, because the changes are so big.
Another question. If everything gets methylated, why is it important to turn off the pluripotency genes in such a complicated way if they get methylated anyhow?

HC: I don't really know the answer to that question, but the problem is that at the time of this de novo methylation, the pluripotency genes are still needed. They get turned off later, after this methylation.

So, for instance, in a mouse – it takes 21 days to make a mouse. The state of de novo methylation grows in about 5 ½ days. The pluripotency genes are turned off later – at about 9 days. Why that is, I don't know, but scientists are not supposed to ask why!

Epigenetics and chromatin

UD: Adrian Bird wrote that "epigenetics is a useful word if you don't know what's going on. If you do, you use something else." Very similar to what you said last time

HC: Adrian Bird made a couple of contributions. One was the discovery of CpG islands. The word "epi" means "on the side". If you have the center of something, the epi-center is to the side of that. In terms of the epigenetics – I don't know what it means.

UD: The term epigenesis was originally used in the 17th century for the Aristotelian idea of emergent development. It meant something after the origin, referring to genesis as "origin", not to genetics. Epigenetics referring to genetics was introduced only in 1942.

HC: It's interesting what Google says about "epi". Sometimes I go to meetings and they argue about this endlessly.

UD: Do you think the term "epigenetics" now is used to get more funding?

HC: Yes, I do it myself! Scientists will do anything to get money! Aside from the philological meaning of the word, there is a concept of epigenetics that is misused greatly. In my mind it should refer to the idea that you can change the expression of something without changing the DNA sequence. That makes something epi-genetic. You can affect something without changing the text.

UD: But the enzymes which bring the methyl groups or take them away …

HC: They're genetic.

I'll give you an example of what I think is an epigenetic phenomenon. Children that are born to mothers who have diabetes during their pregnancy. And then those children are affected at a later age. To me that's an epigenetic phenomenon. I don't know if methylation is involved.

So it's a hard concept to define, but there is something there. Maybe we're making a mistake to use it – I don't know.

UD: Is there a network of the different kinds of epigenetic research and researchers – DNA methylation, histone modification, chromatin remodeling? Is it like in early molecular genetics – there was a group of different researchers who met at conferences to develop the new field?

HC: Yes, sort of. Basically, it's the field of chromatin. It was the same concept. You have the text and the text can be influenced by the structure around it, which is chromatin. And so that's what unites all the different forms of epigenetics. Histones, histone modifications and different proteins that bind to DNA, the structural aspects of chromatin.

UD: But the interaction between researchers - is it among people who do only DNA methylation or who include it in …

HC: Include. Most people who work on DNA methylation are also interested in chromatin structure. DNA methylation probably works

through chromatin structure. It affects how genes are read by affecting the protein structure around the gene.
That, I think is a general concept in biology – that what controls whether the genes are on or off to a large extent controls the availability or accessibility of the gene. This gives a picture of a nucleus with lots and lots of DNA, most of it protected by protein. As a result, the machinery that reads the genes, RNA polymerase, has no access to most of the nucleus because the DNA is heavily covered and hidden under the protein structure. But there are places in the nucleus that are more accessible and more open. That, in a sense, controls what's going to be read. Setting up that protein structure is a complicated thing that we know very little about. One of the things that controls that structure is DNA methylation. So it's a very close relationship between chromatin structure and DNA methylation.

UD: So maybe the future term is "chromatin research"?

HC: Yes, it could very well be.

We did an experiment a long time ago – I think I told you about it last time - Gary Felsenfeld, I and Richard Axel; we were all together in 1971, '72: we took chromatin from blood cells, chromatin, not just the DNA.

UD: You took chicken blood cells?

HC: Yes, chicken blood cells. We first took the DNA from blood cells and liver cells. In a test tube we added RNA polymerase. And we asked, "what's read"? It doesn't matter whether you take blood DNA or liver DNA, or skin DNA, you always get the same thing. Then we took chromatin from a liver and chromatin from a blood cell and put that into a test tube and added RNA polymerase. Here we got globin being made from the blood cell and albumin being made from the liver cell. From the blood cell we didn't get any RNA for albumin; and in the liver cell we didn't get any RNA for globin. That showed – it was the first demonstration - that chromatin provides accessibility to

genes - that basically controls what's read.

A year later, Howard Weintraub – he didn't use RNA polymerase, he used an enzyme that digests DNA – showed that in the blood cell chromatin the globin gene is more sensitive to digestion, meaning it's more accessible. And in the liver cell, the albumin is more sensitive. So that was an addition to this concept of accessibility.

It's fun talking about. We were a great team! In those days we had different instruments. One of the major instruments was this machine for counting radioactivity. We used to prepare the samples and put them into the machine, and then the three of us would stand around. If there was a lot of radioactivity, the numbers of this red, digital indicator would climb up to 1000 or 2000 or 5000 very quickly. If there was very little, you'd see 10, 11, 13 on the meter. We used to stand there for an hour watching the sample go through. For example with this globin experiment. We looked at the globin from the blood cell and all of a sudden we got this big peak of activity – it was amazing!

Immunology

UD: I understand that one focus of your research is on the mechanisms related to DNA demethylation that remove the barriers to transcription in immune cells. How did you become interested in the immune system and how is this removal of the barriers controlled?

HC: I got interested in the immune system because somebody here at the medical school works on the immune system - Yehudit Bergmann. We often talk to each other and in conversations together we thought it would be interesting to look at the role of methylation in the immune system. Here, the situation is different than in a lot of other places. Because in most places, when you take away methylation it allows expression. One of the things that the immune system does is recombination. So we can make hundreds of billions of different antibodies. Up to 10^{11} (ten to the 11^{th}). One hundred billion different antibodies. And the way you do it is by taking pieces

in the DNA and recombining them – making different combinations for different genes, which will make a different antibody.

UD: Is it a stochastic process?

HC: It is largely stochastic. One part of it is not; Yehudit and I work on it and it's absol**UD**ly fascinating to another subject, not methylation. Anyhow, what happens in this system is that DNA methylation controls the recombination. Without taking away methylation, you can't recombine – you can't make these different antibody genes. We showed that you need the DNA demethylation.

UD: Is this demethylation controlled by master genes?

HC: The technical question of what actually gets rid of the methyl growth is basically as yet unsolved. The biochemical mechanism of getting rid of methylation – we don't really know. We have a feeling for it, but a final mechanism we don't have. But what factors in the cell direct this machinery to do the demethylation – that we do have a feeling for. Yehudit and I actually showed that this is done by a very important immune factor called NF kappa B. So we know a lot about the molecular biology. It's very interesting – the effect of methylation here is even bigger than the effect of methylation on transcription. Without getting rid of those methyl groups, you get, basically, no recombination.

But what's really interesting about this is that it only occurs on one allele. Yehudit and I figured out why. That doesn't have to do with methylation. It's a whole different mechanism.

UD: The whole immune system is so complicated anyhow.

HC: It is, it's very complicated and I have to tell you that I've been working with Yehudit now almost 20 years and I still don't understand the immune system – it's just too complicated.

I'm telling you that to emphasize the importance of collaboration. I'm

incapable of learning the immune system like she knows it.

HC: In my life, I've had three wonderful collaborations – one was with Yehudit, one with Aharon Razin, and the other was with Menashe Marcus. Menashe was probably the best geneticist in Israel; he died at the age of 38 of a brain tumor. He was at Hebrew University.

I like the concept that the discoveries of Watson and Crick should be the language of DNA and I like to connect epigenetics with the stuff that we do. That every language has a system for annotation. It is kind of a Talmudic concept. Every language allows the text to be annotated. And DNA is no exception. And we do it all the time – we take a text to read and make marks on it on how we want to read it. And DNA is the same way. It's a natural part of a vast text. The more advanced the text is, the more types of annotations make the text more flexible.

UD: Thank you so much for your explanations and for sharing your thoughts with me.