

Primary Literature Portfolio

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Sodium dichloroacetate (DCA) reduces apoptosis in colorectal tumor hypoxia

Sodium dichloroacetate (DCA) reduces apoptosis in colorectal tumor hypoxia

The title was short and explains what study will be about; however, the first question will come to the reader mind is what about the other kinds of tumor? The study was established by Shahrad and his colleagues (names provide in end) at Department of Biomedical Sciences at University of Guelph in Canada (Q1, 2).

The abstract was well written, which provide the author hypothesis about examining the effect of hypoxia on apoptosis of human colorectal cancer (CRC) cells in vitro and in vivo, which was interesting to compare between them. They provide short summary about the results of the study. However, they failed to describe the experimental design in the abstract. The author used different resources in order to present his point of view (Q3, 17)

There is a relatively low rate of glycolysis followed by pyruvate oxidation in mitochondria in normal cells. However, cancer cells have high rate of glycolysis, which is followed by fermentation of lactic acid in cytosol (Warburg effect). The disorganized angiogenesis of cancer cells may cause hypoxic condition, which will provoke glycolysis and hence accumulate lactic acidosis.

Sodium dichloroacetate (DCA) is a drug that has been used to treat lactic acidosis (low PH in blood or tissue caused by hypoxia). Many studies suspected that the accumulation of lactic acid might contribute to drug resistance in cancer. Thus, attention has been given to DCA as a possible treatment for cancer since 2007. In vitro studies showed that only cancer cells but not

normal cells were killed by DCA. Although this drug has had great attention in curing cancer since 2007, up to date no one has tested it on colorectal cancer (CRC). In this paper, Shahrzad and others hypothesized that this drug might have different effects based on different levels of oxygen (Q5).

Shahrad and his colleagues subjected normal fibroblast and different CRC cell lines (Caco-2, DLD-1, LS174T, SW480 and HCT116) to normoxia, DCA, anoxia and both (DCA and anoxia). Using flowcytometry, they counted the apoptosis rate for each cell line and under each of the four conditions. DCA did not have any effect on the normal fibroblast apoptosis; however, under normoxic condition, DCA had an effect with increasing apoptosis in Caco-2 and SW480 but had no effect on the other cell lines. Under anoxic condition, all the CRC cell lines showed significant increase in apoptosis. Interestingly, under anoxic conditions and in the presence of DCA all the cell lines had lower apoptosis rate than the apoptosis under anoxia by itself. This means that DCA reduces cell death rate under anoxic condition. Furthermore, the author extended his experiment to see the effect of DCA after 48hrs recovery in DCA-normoxic condition. They expose SW480 and Caco-2 to normoxia, anoxia, DCA and both (DCA with anoxia). In each cell line, each condition was processed by two ways. The first way was to expose cells to each factor for 48hrs. The second way was to expose the cells to each factor for 48hrs plus 48hrs recovery in normoxic condition in the presence of DCA. Also using flowcytometry, all cells showed increased apoptotic rate after 48hrs recovery in normoxic condition with the presence of DCA.

The author and his colleagues used western blot to assess the activity of caspase-3, which plays an important role in apoptosis, in 4 different CRC cell lines (SW480, Caco-2, DLD-1 and LS174T). In the presence of DCA + normoxic condition there was a significant increase of caspase-3 activity in SW480 and LS174T while other cell lines are not affected. In anoxic condition, there was an increase in caspase-3 activity in all cell lines. However, adding DCA in anoxic condition reduces all cell apoptosis in all cell lines except DLD-1.

Furthermore, also using western blotting the author and his colleagues assess the level of HIF-1 α (which has a role in apoptosis in hypoxic condition) in 4 different conditions: normoxia, DCA, mimic to hypoxia using CoCl₂ and both (DCA and hypoxia). They used 5 different CRC cell lines (Caco-2, DLD-1, LS174T, SW480 and HCT116). Lamin was used as a loading control. DCA showed no effect on HIF-1 α under normoxic condition; however, it reduces HIF-1 α under hypoxic condition in all cell lines.

Another experiment was done to assess the activity of AKT which is a protein kinase that plays a role in cancer development by protecting cancer cells from P53 induced apoptosis. The author and his colleagues used CRC cell lines (Caco-2, DLD-1, LS174T, SW480 and HCT116). All cell lines were exposed to normoxia, DCA, anoxia and both (DCA and anoxia). Under anoxic condition, AKT was activated, or in another words phosphorlated in all cell lines except HCT116. Surprisingly, DCA activated AKT in normoxia (compared to normoxia by itself) and anoxia (compared to anoxia by itself) in all cell lines except SW480, which did not show any activated AKT under all conditions.

Moreover, the author and his colleagues did an experiment on rats. They injected SW480 and LS174T into immune deficient rats. They waited until the tumor size became more than 35mm^3 . The rats were divided into two groups where one group received DCA and the other one received just water. Treatment was water-containing 1mg/ml of DCA. The tumor size was measured after two weeks for SW480 injected mice and nine days for LS174T injected mice. For the SW480 injected mice, they found a significant increase in tumor size for those rats treated with DCA. However, for LS174T injected mice, there was no significant difference between the treated group and control group.

The author and his colleagues further investigated the SW480 injected tumor. By using the dual immunostaining for CA-IX and TUNEL they were able to compare the necrosis in the DCA treated tumor and the control (untreated tumor) and they found that there was no significant difference. However, when they apply hypoxia versus normoxia they found that there was significant decrease in apoptotic nuclei under hypoxic condition in the DCA treated sample but not in the control. This supports the results of all the previous experiments and highly suggests that DCA might protect cells from apoptosis under hypoxic condition and in the presence of DCA.

Based on these results, we can conclude that DCA has different effects on different cell lines, which means if they move on to human trails, the effect of this drug might differ from patient to patient. Also, we can notice the negative impact of DCA on anoxic condition. Talking about tumors, the center of the tumor will have lower oxygen than the peripheral of the tumor.

Therefore, the DCA will have a negative impact on that tumor as we saw in the SW480 xenografted mouse. Another proof for the negative effect of DCA on apoptosis under hypoxic condition was done through TUNEL staining which showed less apoptosis in hypoxia which supports the cytoprotective effect of DCA under hypoxic condition (Q7-15)

The paper was well written. The authors were successful in providing experimental evidence to answer their main objective, which was proving the fact that DCA has different impacts under different oxygen levels. However, more questions have developed such as, what if they used this drug in combination with 5-fu? 5-fu is a treatment therapy for colorectal cancer patients right now and using it will be another control of effective drug when used alone. Another question is, why did the authors use the term hypoxia in the title but in most of the experiments they applied anoxic condition? This could be misleading for the reader. In the western blot for HIF-1 α , a lamin was used as a load control and one band of this loading control was almost not there. I believed that they should have repeated processing this gel again since the internal control was not perfect.

Moreover, the very idea of decreasing apoptosis in hypoxic conditions by DCA is no more tenable, knowing the fact that cells may undergo several death pathways. Autophagy is a well-known cell death mechanism known to be induced under hypoxic conditions. Authors have made no reference to this important pathway and the probable effect that DCA might have on autophagy before concluding its effect as an anticancer drug. A simple experiment of carrying out LCIII western blot would have taken this into consideration. Therefore, I would asked the

authors for revision even with their convincing result in order to answer my previous questions to make their study successfully achieved (Q4, 18).

References:

Shahrzad, Siranoush, et al. "Sodium dichloroacetate (DCA) reduces apoptosis in colorectal tumor hypoxia." *Cancer letters* 297.1 (2010): 75-83.

Additional resource (16)

Michelakis, E. D., L. Webster, and J. R. Mackey. "Dichloroacetate (DCA) as a potential metabolic-targeting therapy for cancer." *British journal of cancer* 99.7 (2008): 989-994.

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Siranoush Shahrzad, Kristen Lacombe, Una Adamcic, Kanwal Minhas, Brenda L. Coomber.

Characterization of Biofilm Formation by *Borrelia burgdorferi* In Vitro

Characterization of Biofilm Formation by *Borrelia burgdorferi* In Vitro

This paper is an enormous success that provides a better understanding of *Borrelia burgdorferi* the causative of the Lyme disease. We can understand from the title that *Borrelia burgdorferi* has the ability to form a biofilm in vitro. The title is clear and appropriate that may make people expect what the paper will be about. I would use “Studying the Biofilm of *Borrelia burgdorferi*” or “Biofilm as a Survival Mechanism of *Borrelia burgdorferi*”. The research was performed by Dr. Eva Sapi and her research team (names provide below) at University of New Haven in the U.S.

The Abstract has specific information about *Borrelia burgdorferi* and it represents them in the correct form. The hypothesis was mentioned in the abstract which is aggregates that formed by *Borrelia burgdorferi* are biofilms, structures whose resistance to unfavorable conditions.

They then support their hypotheses by providing a short summary of the result. The abstract will be perfect if they provided the experiment design. Also, It will be interesting if the paper has examine the biofilm in the vivo. I would ask if the *Borrelia burgdorferi* form the biofilm just only in an unfavorable condition and when that condition gone it return to the spirochete form. Also, is making the biofilm form will consume more energy of *Borrelia burgdorferi* than if it is in a spirochete

Lyme disease is a result of the different morphological forms of *Borrelia burgdorferi* - spirochetes, cysts, granular forms and biofilms. This morphological of the *Borrelia burgdorferi* were the reasons beside others reason to make Dr, Sapi her research team to assume that *Borrelia burgdorferi* has ability to form the biofilm. They were able to design the experiment and do some experiments that provide there hypothesis.

They used different resources and references to collect the whole information about *Borrelia burgdorferi* which provided enough information about *Borrelia burgdorferi* to establish and design their experiment (Q1-5).

They used different experiments and as result they got their convincing result. In order to know the different stages of *Borrelia burgdorferi* they used different modes of microscopy. Also, they used the AFM to have better understanding about internal morphological that rearrangement during *Borrelia burgdorferi* aggregate development. Studying the extracellular polysaccharides on the surface of the *Borrelia burgdorferi* aggregates were important too; therefore, they used the Spicer & Meyer sequence in order to differentiate between non-sulfated/carboxylated and sulfated mucins. Moreover, the experiment repeated using immunohistochemical methods with anti-alginate and anti-*Borrelia* antibodies to measure the substant that cover the aggregates. Using negative control samples were important to support the result; therefore, the primary antibody was omitted from slides with *Borrelia* cells and aggregates and replaced with normal rabbit sera. Moreover, Calcium presence on the surface of the *Borrelia burgdorferi* aggregates were examined using a calcium specific staining method with Alizarin Red-S stain and analyzed by different microscope. Also, Extracellular DNA that may presence on the surface of the *Borrelia* aggregates was observed using nucleic-acid-specific dyes. All these experiments were able to provide enough evidence that *Borrelia burgdorferi* can form a biofilm.

In the result section, the figures were presented and explained clearly. They provided which kind of microscope did they use, and in which magnifications. Also, they mentioned which cultured did the use to take the pictures. The results were convincing after comparing the author hypothesis and their result. Pictures were demonstrated the biofilm of *Borrelia burgdorferi* clearly and they used different microscopes and dyes to prove their hypotheses which successfully achieved. However, I was wondering if the pictures in the Fig.1 were taken by using the same cultures (the same tube)? Or only same strain of *Borrelia burgdorferi* (B31-B31-GTP) but different tube?

Also, for the pictures in Fig.1 the one that were taken in the early stage (0-2 days of aggregation), they used Dark filed and DIC microscopes in (A and D) but why I feel they used different sample? It will be better if the used the same picture when they took it by dark filed microscope and then take it by DIC; then compare them. The same thing when they did in Fig.5, they used same sample in different microscope. They, however, used Spicer and Meyer stain there.

In Figure 2, the pictures were presented in clearly and in a nice way, the arrangement of the pictures help the data to be understated easily. Using the AFM were an advantage to support their data. They start taking pictures after 2, 4, 6, 12, 14, and 21 days of culturing. It was obviously how the *Borrelia burgdorferi* aggregate and start to form the biofilm. However, I was surprising with the empty space when they start to aggregate (circular space), it started in B and were clear in C. In D, there was one big empty circle, why this is happened and is there any reason to make the spaces or just simply this is how *Borrelia burgdorferi* making biofilm. This circle was also in E and small in F (located in 9 clock area).

Using Software was also another advantage to support their hypothesis and clarified their data easily as we can see in Fig 3-4. They used the AFM to take pictures of *Borrelia burgdorferi* B31 Strain after 20 days of culturing, and then they provided 3D AFM images of B31 strain in the early aggregates on agarose substrate.

In discussion part, they explained the whole results in details that supporting their hypotheses, it was short and in completed form. We know that journals have their own design for writing the paper, but it would be more organized if all figures were provided before providing the discussion part. For example, Figures 5-8 were in the middle of the discussion part.

The method and materials were explained the experiment and provided enough information to be repeated again from any person. Not only this but they organized every experiment and explained what they did.

The most part that I like is how they presented the pictures in different kind of microscopes. It helped me to compare and observe the biofilm clearly. Also using the software to demonstrate some of pictures help the reader to easily understand the morphology of biofilm. In general the paper was well written and easy to understand, and reader will not need to another resource to about the topic to understand the paper. Therefore, I would accept the paper if I were a reviewer as a result of the enough evidence that proved their hypothesis (Q6-18).

Reference:

Sapi, Eva, et al. "Characterization of biofilm formation by *Borrelia burgdorferi* in vitro." *PloS one* 7.10 (2012): e48277.

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The human translation initiation multi-factor complex promotes methionyl-tRNA_i binding to the 40S ribosomal subunit

The human translation initiation multi-factor complex promotes methionyl-tRNA_i binding to the 40S ribosomal subunit

We can learn from the title that what the authors want to emphasize, which is human translation initiation multi-factor complex, promotes methionyl-tRNA_i binding to the 40S ribosomal subunit. This study was conducted by Masaaki Sokabe, Christopher Fraser, and John Hershey at Department of Biochemistry and Molecular Medicine and Department of Molecular and Cellular Biology at University of California in the U.S. The abstract has descriptive information about the authors' hypothesis and the experimental design. In this study, they used different resources related to the topic to support their point of view (Q1-3).

Initiation of protein synthesis in eukaryotes requires the recruitment and positioning of the initiator tRNA on the small ribosomal subunit. The 40S subunit recruits tRNA in the form of a ternary complex (TC), which consists of eIF2-GTP-Met-tRNA. In addition, other eukaryotic initiation factors (eIF1, eIF1A, eIF3 and eIF5) facilitate stabilization of the TC on the 40S subunit forming a 43S pre-initiation complex. The mRNA is then associated with the pre-initiation complex allowing it to scan and recognize the start codon. When the AUG start site is recognized, eIF5, a GTPase activating protein, promotes hydrolysis of eIF2-GTP releasing a phosphate that dissociates through eIF1. This places Met-tRNA_i in the P-site and releases eIF2-GDP. This form of eIF2 is then recycled to its GTP form to be incorporated into another initiation round. Finally, a 60S subunit will join the mRNA forming the 80S initiation complex that is now ready to synthesize the encoded protein (Q5).

Studies in *Saccharomyces cerevisiae* have shown the existence of a stable multi-factor complex (MFC) that contains many initiation factors. Using a pull-down assay it was shown that eIF1, eIF2, eIF3 and eIF5 are found in a MFC both *in vitro* and *vivo* in the absence of the 40S subunit. Further investigation of the cell lysates has shown that the ribosome-free MFC was associated with Met-tRNAⁱ. Moreover, the interaction between the MFC components in yeast was proven to be exclusive. Also, the formation of the MFC plays a vital functional role in protein synthesis. Other studies were conducted to test the characteristics of MFC in other organisms such as plants and mammalian, but mixed results were obtained (Q6).

In this paper, Masaaki Sokabe, Christopher Fraser, and John Hershey asked, if the MFC also formed in humans and mammals? The authors identified a human pre-formed MFC that was able to deliver the initiator tRNA to the 40S subunit. Surprisingly, they also found that eIF2 had the ability to bind directly to the 40S subunit with or without the other initiation factors. The authors were interested in investigating if there are indeed 3 possible pathways for Met-tRNAⁱ-40S binding; either TC-mediated (Met-tRNA-eIF2-GTP), MFC-mediated, and direct in the human cells.

The authors initially aimed to test if free MFC was present in mammalian cells *in vivo*. They subjected HeLa cell lysate and rabbit reticulocyte lysate to native-PAGE. The characteristics of the gel prevent the entry of the ribosomes and their complexes in the gel, thus insuring that the product results are strictly MFC complexes. They then analyzed the results using western immunoblotting against each factor. Purified eIF3 and reconstituted MFC were used with Met-tRNAⁱ as controls. The results showed the presence of all anti-eIFs 3a, 5 and 2 reactive bands within the control ranges in all lanes. This finding led the authors to suggest the existence of MFC in human and rabbit cells that are free of the ribosome.

Furthermore, using the results from the previous experiment, the authors wanted to resolve if the resulting components also contains initiator tRNA. The approach they took was to splice the region of the gel containing the MFC components and subject the extracts to RT-PCR. The outcome that appeared provided the presence of Met-tRNA and that it was indeed bound to the MFC, specifically to eIF2-GTP where it had the highest yield.

Knowing that MFC exists in a free state in human cells, the authors began exploring the ability to form a MFC using purified human initiation factors *in vitro*. They incubated different combination of eIF2 and eIF3, purified from HeLa cells, with eIF1, and eIF5 that were isolated from bacteria. Afterwards, the resulting products were examined using native-PAGE. eIF3 was used in this assay as the standard base to which other factors were sequentially added. The authors were able to see a clear mobility shift in each lane of the results, thus implying the formation of the MFC *in vitro*.

To verify if the human MFC can indeed form *in vitro*; more tests were conducted using the previous result. The shifted band containing the whole MFC proteins were excised and run on an SDS-PAGE. The results supported the previous hypothesis that all the factors were associated with one another. eIF2, eIF5 and eIF3 band appeared to correspond to the controls and eIF1 was detected using immunoblotting.

To gain a better understanding of MFC assembly, the authors looked at the formation of binary complexes between each MFC component. The eIF1 was added at a higher concentration to ensure its binding. The formed complexes were analyzed using native-PAGE, immunoblotting techniques, and SDS-PAGE. The results revealed that the initiation factors affiliated together at various strengths as binary complexes.

As well as establishing the interaction between the human MFC, the authors went an extra step and tested the MFC interaction in yeast using the same techniques. They realized that the interaction between the factors were mostly similar, and with stronger affinity, but not identical to that in humans.

The authors went on to examine the rate at which MFC and TC promote Met-tRNAⁱ binding in humans. Three different Met-tRNA-40S binding reactions were examined. The first was a direct binding pathway where Met-tRNA binds directly to the 40S subunit fixed with (eUF1, eIF1A, eIF2GDPNP, eIF3 and eIF5). Secondly, a TC-mediated pathway with the TC formed and added to the 40S subunit. Lastly, an MFC-mediated pathway with MFC performed with Met-tRNA and GDPNP. A delivery assay was performed after adding the complex to the 40S subunit. The delivery assay was done to detect the rate at which the complexes deliver Met-tRNA to the 40S. The results suggested that after 30min 60% of the input ribosomes bind to Met-tRNAⁱ in both the TC and MFC mediated delivery pathways. However, only 20% binding of Met-tRNAⁱ to the 40S within the same 30min was seen through the direct binding pathway. It was presumed that both TC and MFC stimulate Met-tRNAⁱ binding to the 40S at a similar rate. The authors concluded that "to insure an efficient rate of delivery in this in vitro assay, Met-tRNAⁱ must bind to eIF2 as part of the TC or MFC prior to delivery to 40S subunit."(Sokabe et. AlI, 2011)

Next, the authors investigated the binding efficiency of different MFC components individually to the 40S-eIF1 complex. A native-PAGE followed by western blot analysis was performed. Binding was demonstrated by a band shift analysis of the bands, then using an antibody specific for the initiation factor, that were detected. The resulting data indicated all the

initiation factors were able to bind to the 40S-eIF1 complex stably. Also the factors binding to the 40s subunit precedes Met-tRNA_i binding.

From their study, the authors concluded that because they were able to assemble MFC from purified human proteins, then the MFC can exist free from ribosome, which was interesting. The occurrence of MFC-Met-tRNA_i complex in the cytoplasm of HeLa cells further supports its freeform. The authors were also able to prove that TC and MFC were able to deliver Met-tRNA_i to the 40S subunit at similar rates, suggesting that they both play an identical role in delivery in human cells. The authors also proposed that since the MFC components are able to bind separately to 40S ribosome then the Met-tRNA_i might bind directly to such 40S-factor complex. Finally, the authors combined the results to confirm the three different pathways for delivery of Met-tRNA_i to the 40S ribosome. However, they weren't able to distinguish the dominant pathway in the human cells (Q7-17).

I think that the paper was well written. The authors were successful in providing experimental evidence to answer their main objectives. However, more questions have developed. For instance, in what order do the MFC components form? They could have mixed the factors together and monitored the complex formation in different time's using SDS-PAGE. Could the formation of the MFC be mRNA specific? On the other hand, the authors neglected to study if MFC also delivers the Met-tRNA_i to 40S in yeast, so they couldn't establish if the MFC mediated pathway was universal in eukaryotes. Further studies could have been done to discriminate between the three pathways to decide which is most efficient in human cells. They could further compare the results they derived from the binding rate assay to results from experiments that are based on inhibiting the formation of the MFC or the TC. If the MFC fails to assemble the cell would depend on the TC- mediated pathway. Then they can examine the

results, to see if translation initiation rates decrease or remain the same to a wild type. Therefore, I would ask the author for revision and strongly suggest considering my previous questions in order to accept the paper (Q4, 18).

Reference:

Sokabe, Masaaki, Christopher S. Fraser, and John WB Hershey. "The human translation initiation multi-factor complex promotes methionyl-tRNA_i binding to the 40S ribosomal subunit." *Nucleic acids research* 40.2 (2012): 905-913.

Authors' names:

Masaaki Sokabe, Christopher S. Fraser and John W. B. Hershey.

BRCA1 is a component of the RNA polymerase II holoenzyme

BRCA1 is a component of the RNA polymerase II holoenzyme

We can learn from the title that BRCA1 is a component of the RNA polymerase II holoenzyme, which is simple and can tell the reader what the authors want to prove in this study. This research was conducted by Ralph Scully at Department of Pathology and Women's Hospital at Harvard Medical School in Boston (Q1, 2).

The abstract was written in a good way that explains the hypothesis of the authors and how they design their experiment with providing a short summary of the results. The idea led to this research the fact that BRCA1 is a gene known to have a role in tumor suppression. Mutation of this gene, which results in truncations in most of the cases, had been observed in many breast and ovarian tumors. The mammalian RNA polymerase holoenzyme contains multiple basal transcription factors, and SRB homologues. When the carboxy-terminal segment of BRCA1 is fused to the DNA binding domain of GAL4, it can activate transcription. Moreover, the transcription was inactive when there was point mutation in BRCA1, which indicates that BRCA1 has an important role in transcription. The authors used different resource about BRAC1; however, their point of view was achieved successfully .In this paper, the authors hypothesize that BRCA1 is a part of RNA polymerase II holoenzyme and used a new technique to purify specific transcription factors associated with RNA polymerase II (Q3, 5, 6)

The aim of the study was to determine whether BRCA1 is a component of RNA polymerase II holoenzyme or not. Therefore, the study focused on the interaction between BRCA1 and the holoenzyme complex. It is important to stress that as any other protein, the knowledge of BRCA1 location and interactions are crucial to the understanding of its function. In addition, a part of this study was dedicated to study the transcriptional function of BRCA1. Thus, BRCA1-RNA polymerase holoenzyme complex was tested for transcriptional activity. Moreover, this study also looked at the effects of truncated BRCA1 protein on its interaction with RNA polymerase II holoenzyme. BRCA1 is a tumor suppressor gene, which means there should be at least one wild type copy of this gene in order to prevent cell transformation. So, another important question is whether tumor suppression activity of BRCA1 is related to transcription function or DNA repair activity.

To study the interaction between BRCA1 and of RNA polymerase II holoenzyme, the authors used HeLa cell extract to perform purification and immunoprecipitation.

HeLa cell extracts that go through the Bio Rex 70 column show the presence of a co- elution of BRCA1 and RNA polymerase. This elution clearly appears in SDS/PAGE gel in the specific potassium concentration 0.6 M (KOAc) as seen in Figure 1 part A. RNA polymerase II (pol II) is known to be in the holoenzyme form in vivo. Therefore, we can suggest this question, is BRCA1 able to interact with RNA polymerase II in absence of other holoenzyme components? After centrifugation of 0.6 M elution through sucrose gradient, two peaks were found to contain BRCA1. In Figure 1 part B, One of them has BRCA1 in addition to polymerase II and cdk8 (peak A) while the other only has BRCA1 (peak B). This observation illustrates that only a

fraction of BRCA1 was associated with holoenzyme after sucrose gradient. In peak A, These three proteins were elicited from more than 99% of other protein. In the next step of purification, the researchers took peak A purified protein and ran it through metal chelate chromatography. Again, BRCA1 and polymerase copurified together with one of the cdk8 peaks. There is another peak that showed purification of cdk8 alone, which inspires the authors to suggest that it is a different complex from the one that copurified with polymerase and BRCA1. After this step RNA holoenzyme polymerase reaches about 400 folds purification and still has BRCA1 to purify with. This indicates strong stability of BRCA1- RNA polymerase-cdk8 complex which can be observed in Figure 1 part C.

The authors tried to see if there are other transcription factors that, like BRCA1, purify with holoenzyme. Immunoblotting was done on sucrose gradient fractions by using different specific antibodies. Although YY1 purified at the same level of potassium that holoenzyme did, YY1 did not co-sediment with holoenzyme through sucrose gradient purification. In another matter, to assess the other function of BRCA1 regarding DNA repairing the authors studied BRCA1-Rad51 complex. This complex failed to be purified through sucrose gradient. The authors attribute this failure to two possible reasons, weak stability or low quantity. The authors did not investigate which one is true. They could examine the stability with different concentration gel shift procedure or competitive binding technique, as we can see in Figure 2.

Furthermore, the association between proteins can be studied by immunopurification. The researchers used an antibody against another component of holoenzyme. Anti-hSRB7 was used

on Bio-Rex 70 0.6 M protein fraction. The first fraction was purified and treated with the anti-body against hSRP7, pol CTD, E1A and BRCA1. After purification, each product went through immunoprecipitation with the BRCA1 antibody. E1A was used as negative control since it not a component of human holoenzyme. For further confirmation, they ran immunoblotting of anti-BRCA1 in presence of antigenic peptide that blocks antibody protein binding by specifically competitive binding. The gel shows no band when the BRCA1 antibody has been blocked, which can be exhibited in Figure 3 part A.

The same work was done using anti-pol II, instead of anti-BRCA1, in the immunoprecipitation step. In addition, when anti-pol II was blocked by antigenic peptide, there was no immunoprecipitation band. The gel showed that the quantity of anti-BRCA1 immunoprecipitation that reacted with anti-BRCA1 is higher than the one that reacted with anti-pol. As result of that, the authors suggest that BRCA1 binds to some but not all RNA polymerase, as can be seen in Figure 3 part B.

Another question to be answered is what else, beside RNA polymerase, coimmunoprecipitate with BRCA1? More specifically, are general transcription factors, coimmunoprecipitate with BRCA1? To answer this question, researchers made their case depending on two sub questions. Is the fraction of the pol II that coimmunoprecipitate with BRCA1 is active or not. The transcription activity of BRCA1 immunoprecipitate was examined by G-less cassette technique. Transcription factors were added to mixture but not RNA polymerase. The gel showed transcription activity with both anti BRCA1 C and N terminals. So

the next question was which general transcription co immunoprecipitate with BRCA1 and which did not. Again, G-less cassette technique was used with but one of TFs was removed each time. Removing TFIIF, TFIIE and TFIID did not affect transcription. They could also mutate one of general transcription factors each time in extracts either genetically or specific PH then they added BRCA1 co immunoprecipitate to see if that transcription factors is present and able to perform transcription. When TBP and TFIIB have been removed BRCA1 immunoprecipitate failed to show transcription, unlike TFIIF, TFIIE and TFIIH which, when they had been removed, the complex maintained transcription activity as shown in Figure 4. This observation indicates the presence of TFIIF, TFIIE and TFIIH in BRCA1 immunoprecipitate but not TBP and TFIIB. So, we can ask if BRCA1 binding to polymerase is after TFIIF, TFIIE and TFIIH bind and prior to TBP and TFIIB binding to pol II? Is BRCA1 control when TBP and TFIIB bind to polymerase? In other words, is BRCA1 function is to prevent TBP and TFIIB binding to polymerase until something else happened? These questions are yet to be answered.

The last question the authors ask is what the effect of BRCA1 mutation on its ability to bind the holoenzyme. To answer that, 293T cells were transfected with HA tagged wild type and mutant BRCA1. Immunoprecipitation of holoenzyme from cells extracts was done using hSRB7. For this experiment to be valid, same amount of wild type and mutant BRCA1 should be synthesis by transfected cells. To make sure the synthesis is equal the authors grew the cells in [³⁵S] label medium and then ran their extract on SDS/PAGE gel. The authors also performed immunoblotting by using anti-HA. Anti-BRCA1 western blot showed significant reduction in the ability of mutant BRCA1 to bind holoenzyme in comparison with wild type in Figure 5. The authors smartly chose the mutation that had the minimum number of deleted residues. (Q7-17)

In general, I think that the paper was well written. The authors were successful in providing experimental evidence to answer their main objectives. Their findings support BRCA1 is a part of RNA polymerase II holoenzyme, which is interesting. Their work also demonstrated how this strong association is specific for BRCA1 as many transcription factors failed to co-purify with pol II holoenzyme. Moreover, BRCA1 associated pol II shows transcriptional activity as reported in this study. Finally, truncated BRCA1 failed to interact with pol II holoenzyme. However, the idea of testing their hypothesis on HeLa cells, which is cervical cancer cells, does not seem to be the perfect choice. One can argue that its transcription system was affected because of the cancer. Authors have made no reference to the cancer status of HeLa cells and the probable effect that might have on transcription system before concluding that BRCA1 is a part of holoenzyme in normal cells. A simple experiment of carrying out normal biopsy or normal cell line as a control would have taken this into consideration. Mainly the authors succeeded in answering the main questions. The authors managed to answer questions about BRCA1 function in transcription process. Still it is not answered how it linked to the function of BRCA1 in genome repairing as mentioned in the hypothesis. However, I would accept this paper if I were reviewer for this journal as result of their fundamental discovery.

(Q4, 18)

NF- κ B2 mutation target survival, proliferation and differentiation pathways in the pathogenesis of plasma cell tumors

NF- κ B2 mutation target survival, proliferation and differentiation pathways in the pathogenesis of plasma cell tumors

We can learn from the title that NF- κ B2 target different pathways in the pathogenesis of plasma cell tumors. It would be better if the author name it “characterization of NF- κ B2 mutation in plasma cell tumors”. The study was performed by Brian A McCarthy and his colleagues at Cancer Center and Department of Pathology, Medical college of Georgia (Q1-2).

The abstract had a clear background summary of the NF- κ B2 mutation, method, and result of the study. The author presented his hypotheses clearly with a short description of the experimental design. In this paper, the author and his colleagues used different resources and earlier studies related to NF- κ B2 mutation in order to present their point of view, which is providing the first direct evidence for a causal role of NF- κ B2 mutation in the pathogenesis of mouse plasma cell tumors that share some key histopathological and clinical features of human MM (Q3, 6).

Cancer is a disease of uncontrolled cell growth, which known to be monoclonal source. This elevation in number of one cell type over others affects the normal ratio of different types of cells that build up body tissues. Cancer cells not only cause symptoms in their immediate surroundings but also affect other body systems. There are many ways that allow cancer cells to develop. For example, interrupt cell signaling programs by truncated receptors or transcription factors. These impair proteins which lose their activation binding domain usually provide constitutive signaling. Studying these changes not only allows scientists to understand the

molecular process of particular cancers, but also offers new treatment targets. These facts about cancer were the main reason that led to this study (Q5)

NF- κ B2 is a transcription factor that phosphorylates by cytokines. This allows the mature NF- κ B2 to dimer with Rel proteins. This dimer then binds to DNA on κ B sites and promotes important genes that control proliferation and differentiation pathways. The constitutive activation of NF- κ B2 may occur by chromosomal translocation which is found in different lymphocyte tumor. Multiple Myeloma (MM) is a common, incurable malignant tumor of plasma cells.

The purpose of this study is to determine the effect of NF- κ B2 mutation as a major cause in developing plasma cell tumors. Furthermore, this study aims to understand the molecular mechanism behind MM tumorigenic process. To do this, the authors studied the development of transgenic mice p80HT. These mice had the earlier event in the lymphocyte tumors development process (p80HT) in addition to NF- κ B2 mutation. According to previous studies, the authors mentioned that the expression of p80HP by itself without NF- κ B2 activity can induce little up regulation in many important genes to reduce apoptosis of T and B lymphocyte but over long period. Therefore, the authors suggest that another genetic change is required to transform plasma cells into malignant tumors.

p80HP transgenic (NF- κ B2 active) mice would develop a variety of B-lymphocyte tumors.

However, the authors mentioned that in a previous study, histological examination of 12 samples showed that half of them (6/12) were plasma cell tumors. Biopsies were fixed with formalin, embedded in paraffin wax, and then sections were taken and stained with Hematoxylin and Eosin

(H&E). Histological examination is very useful as a first step to scan and classify tumors as we can see in Figure 1. Even though cancer cells undergo many genetic alterations, they still retain some of the morphological criteria of the origin tissue. In addition, many tumors show a specific Cytoplasm/Nucleus ratio. In Figure 1 b, histological results were confirmed by flow cytometry and immunohistochemistry staining for CD138, a marker for plasma cells, which showed accumulation of plasma cells in tumor sites (>10%) in comparison with wild type (<2%).

In this study, serum protein electrophoresis was performed on 25 mice. Electrophoresis gel displayed M spike band in 40% of p80HP transgenic mice. Ten mice showed Monoclonal Immunoglobulin (M protein) positive at the age of one year. Some of these mice also showed the common manifestations of human MM. The number of plasma cells was found elevated to represent more than 10 percent of total cells in bone marrow, which can be observed in Figure 2. In the same Figure, X-ray examination indicated the presence of lytic bone lesions. In addition, bone density decreased in comparison with wild type littermates. The bone sample was taken from the femur and spine area to analyze 3D morphometric parameters by CT system. Three out of ten M-protein-positive mice were sacrificed in order to perform a gene expression profile and what remain of the seven were kept to observe plasma cells tumor development. The authors used transgenic p80HT mice splenic B cells to study gene expression. They focused on B cells based on the reasoning that identification of genes activated in these plasma cells precursors might shed light on the molecular and cellular process that drive the development of plasma cell tumor. Wild type littermates were used as standard in gene expression profiling, disregarding to individual differences. The seven remaining mice have developed plasma tumors at the age of 1.5 years.

The gene expression profile showed significant change in quite high number of genes. As many as 201 genes were either upregulated or downregulated (± 1.5). These genes were analyzed by using gene annotation enrichment analysis data bases to look for Gene Ontology (GO), which can be seen in Figure 3. GO confirms the presence of genes across species as well as their biological role. These analyses revealed a network of p80HT target genes with functional significance in pathogenesis of MM by regulation of differentiation, Proliferation and apoptosis of plasma cells and/or MM cells.

The role of NF- κ B2 was found to be important in the development of plasma cell tumor by interacting with many signaling pathways. In table 1, IL-10 is one of the top 5 upregulated gene and one of target genes of p80HT. This gene product is very vital in cell proliferation and survival. Also, IL-10 play a role in cells differentiation by interacting with other gene called CD27. Furthermore, IL- 10 pathway needs Stat3 activation to up regulation expression of Cyclin D including Cyclin D1, Cyclin D2, Survivin, and Blimp1. Those genes were having clear elevation in gene expression microarray. Moreover, RT-PCR used to confirm up regulation of CD27, Cyclin D1, Cyclin D2, IL-10, IL-15 and Survivin. Microarray showed another effect of active NF- κ B2 which is the upregulation CD30 and CD 30L which are work together to increase cell proliferation.

Importantly, the authors established a novel link between NF- κ B2 and IL-10. To prove this link, they used three human MM cell lines which have different NF- κ B2 Activities. These cell lines were treated with anti-stat3 (S3I-201) which inhibits DNA-Stat3 binding by preventing Stat3

phosphorylation. Immunoblotting as well as quantification of cell growth and death (cell viability count) were used to evaluate the effect of anti-stat3 on previously mentioned cell lines.

In Figure 4, Immunoblotting was performed by using primary antibody against stat3 as well as other gene product the affected by IL-10-stst3 signaling pathway including Cyclin D1, Cyclin D2, and Survivin. B-actin was used as positive control, and it would be better if they include a negative control as well. They also used secondary antibody conjugated with chemiluminescence in order to make the bands visible. In the absence of S3I-201, the gel showed high presence of phosphorylated stat3 (pStat3-Y705) in the high NF-kB2 activity (EJM) cell line, while the low NF-kB2 activity (H292) showed less phosphorylation. On the other hand, the interim NF-kB2 index cell line 8226 showed no presence of pStat3-Y705 at all. After cell lines treated with 50 μ M Anti-stat3 for 24 hours, the expression of Cyclin D1 and Servivin decreased in both EJM and H292 cell lines whereas 8226 cell line show no changes. In a quantification study of cell growth and death, the 8226 cell line was resistant and show apoptosis concentration as high as 200 μ M. On the other hand, both high NF-kB2 activity (EMJ) and low NF-kB2 activity (H292) cell lines showed sensitivity to S3I-201. Approximately 90% of EMJ cells line which have high, lost viability in the presence of 50 μ M S3I-201 within 2 days, whereas H292 cells showed only 40% of cell death under the same condition. This difference in sensitivity indicates the effect of NF-kB2 activity on the IL-10-Stat3 signaling pathway. Cells with high NF-kB2 activity have a high active IL-10-Stat3 signaling pathway that makes them more susceptible and sensitive toward anti-stat3 (Q7-15)

Further evidence of NF- κ B2 lies in the up regulation of one of its targets, IL-15. IL-15, the second upregulated gene (+ 4.57 fold) was reported to have autocrine loop activity in plasma tumor cells. This study shows not only an increase in transcription of IL-15, but also in its receptor IL-15Ra (+ 1.7) in NF- κ B2 mutant p80HP transgenic mice. This finding indicates the importance of NF- κ B2 mutation in MM pathogenesis, since IL-15 enhances MM cell proliferation.

Based on previously mentioned findings, the authors concluded that NF- κ B2 has causal role in the development of plasma cells tumor. NF- κ B2 transcriptional activity induces proliferation and survival through activation of important set of genes including IL-10, IL-15, Cyclin D1, Cyclin D2 and Survivin.

I think this study provides clear evidence of NF- κ B2 role. The authors used a variety of techniques in this experiment to confirm the presence of characteristics of human MM in mutant p80HP transgenic mice. They also confirmed their gene expression data with RT-PCR, which make it stronger and interesting about this study. The authors pointed out those three mutant p80HT transgenic mice used for gene expression microarray did not have plasma cell tumors and could not be guaranteed to develop plasma cell tumors. As result of that, it is reasonable to ask why they did not perform gene expression microarray on mutant p80HT transgenic mice that already developed plasma tumor as well? The relationship between p80HT and NF- κ B2 needs more clarifications. In addition, the authors mentioned that the immunoblotting showed

a decrease in the expression of Cyclin D1 in EMJ cell line after treating with anti-stat3 but the gel showed no expression of Cyclin D1 in the absent of anti-stat3. Also, the paper has many tables that could mislead the reader. Therefore, it would better if the authors complete their paper with following these suggestions, however, the result in general were convincing, and I would publish the paper if I were a reviewer of this journal (Q4, 17, 18).

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Additional resources (16)

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Authors' names:

Brian A McCarthy, Liqun Yang, Jane Ding, Mingqiang Ren, William King, Mohammed ElSalanty, Ibrahim Zakhary, Mohamed Sharawy, Hongjuan Cui and Han-Fei Ding.

Design and Performance of the CDC Real-Time
Reverse Transcriptase PCR Swine Flu Panel for
Detection of 2009 A (H1N1) Pandemic Influenza
Virus

Design and Performance of the CDC Real-Time Reverse Transcriptase PCR Swine Flu Panel for Detection of 2009 A (H1N1) Pandemic Influenza Virus

We can learn from the title that author and his team were able to design and perform CDC Real-Time Reverse Transcriptase PCR Swine Flu Panel for detection of 2009 A (H1N1). The title is quite long, and it would be better if the name it "CDC real time transcriptase PCR of H1N1".

The abstract of this paper is well written. This is because the person is able to familiarize himself/herself with the content of the paper. It is also written in an effective language, which the reader is able to read easily. The terminologies used are well symbolized, which avoids repetition of words by using the symbols. This creates an attracting feature of the paper, which enables the reader to have an easy time when reading the paper.

The abstract provides the reader with a quick hint of the swine influenza viruses (SIV) and real-time reverse transcriptase PCR (rRT-PCR) procedures. As such, the reader has the ability of linking between the previous rRT-PCR procedures and the current rRT-PCR procedures. Further, this summary enables one to identify the results, which were obtained by the CDC rRT-PCR Swine Flu Panel. In addition, the summary creates awareness of the role and distribution pattern of the CDC rRT-PCR Swine Flu Panel. This is because the reader is able to identify that CDC rRT-PCR Swine Flu Panel was a timely and specific tool, which was used in 2009 to detect A (H1N1) pdm influenza viruses. The study was done by Bo Shu and his colleagues (names provided in end) at Georgia in the U.S. (Q1, 2, 3, 5).

The introduction of the paper is concise and comprehensible. This is because the introduction enables the reader to have background information on the experiment, which is being conducted. This introduction includes the definition of swine influenza, the origin of swine influenza, the detection periods of swine influenza, the spread of swine influenza, and the victims of swine influenza. In this, swine influenza seems to affect pigs, and then humans are the second vulnerable victims. This background information creates room for the reader to predict the anticipated results of the experiment and this reason that led to this research. This research was based on previous studies for swine influenza and they used many resources to establish their experiment (Q6)

The experiment was based on CDC procedures, and it was conducted by CDC rRT-PCR Swine Flu Panel. On the same note, the introduction gives background information on previous CDC rRT-PCR procedures, which had been conducted, as well as the results. This creates a room for the reader to link between the previous studies and experiments and the current experiment, which is presented in this paper. The presentation of the experiment in the introduction section enables the reader to have an able time while reading the rest of the paper. This is because the reader is able to develop a background picture, in mind, on the experiment, which was conducted.

However, the introduction of this paper fails to outline the objectives of the experiment effectively. This makes the reader read deeply in order to comprehend the main aim of the experiment. This is an effective way of ensuring that the reader does not just skim the paper, but

reads comprehensively in order to understand the main focus of the paper. In addition, the paper does not clearly outline the importance of the experiment. As such, one is left to read deeply in order to gather adequate information on why the experiment was conducted (Q4, 7)

The method section of this paper is informative. This is because it has the ability of informing the reader of the experiment, which occurred. In this, the method section outlines the various materials, which were used in the experiment effectively. This outline includes information on where such materials were obtained, how the materials were obtained, and conditions of these materials before and as they being used in the experiment. These materials include clinical specimens and influenza viruses, RNA extraction, CDC rRT-PCR Swine Flu Panel probes and primers, and rRT-PCR.

Such information, which was included in the methods section, is crucial to the reader in case the reader has desires of replicating the work, which was performed. Further, the individual who conducted the experiment has the potential of repeating the experiment in order to see whether the initial results are attainable after the repetition of the experiment. This is possible since the method section of this paper provides adequate details, which have the impact of affecting the overall performance of the experiment, when repeated, in the future. This was evidenced by the presentation of rRT-PCR conditions for the rRT-PCR reactions.

However, the method section of this paper would have been eye capturing if visual aids such as photos of the materials were used. This would have aided the reader to identify effectively the various materials used in the experiment (Q4, 7).

The presentation of the results in this paper is effective and attracting. This is because the results are presented while using visual aids such as charts, figures, and tables. This enables the reader to focus effectively on the data, which was obtained after conducting the experiment. Further, the organization of the results according to various subtopics, aids in effective comprehension of the obtained data. These subtopics include CDC rRT-PCR Swine Flu Panel reaction efficiencies, analytical sensitivity, analytical specificity and inclusivity, and CDC rRT-PCR Swine Flu Panel clinical performance monitoring. Such an organization of results presentation enables the reader to comprehend clearly the data, which was obtained after the performance of the experiment. Furthermore, this form of results organization facilitates in effective analysis of the results obtained. Moreover, the results are presented in a manner that is related to the main focus of the experiment, which was performed.

In Figure 1, the authors represented the genotypes of N. Am tr-SIV H1N1, H1N2, and H3N2 subtypes and 2009 A (H1N1) pdm influenza viruses. It has different color and was well organized. On the other hand, Figure 2, representing the rRT-PCR and genetic sequencing result versus the number of the specimens. However, I was wondering according to what they collect the specimens? Was there certain age? Or just that patient is a positive for the infA and swInfA but negative for swH1.

In Figure 3, the author show the locations where nucleotide differences were observed are indicated after doing nucleotide sequence alignment of swH1 primer and probe regions from

specimens and of viruses positive for 2009 A (H1N1) pdm influenza virus that tested negative by the swH1 assay (Q8, 9)

The discussion of the results on this paper is admirable. This is because the discussion involves analyzing of the results obtained, as well as focusing on a brief discussion of the results implications. However, the discussion of the results in this paper failed to identify errors, which might have emerged during the performance of the experiment. As such, validation of the conclusion, which was developed after the performance of the experiment, is questionable. This is because, in each experiment, at least one or two errors might occur during the whole process of the experiment or partial process of the experiment. Therefore, there was a need for the discussion, which was adopted in this paper to acknowledge the presence of experimental errors, as well as the impact of the errors in making the conclusions of the experiment.

This study is critical since it gives adequate information on the origin, evolution, and spread of the influenza virus. The study also identifies factors, which contributes towards the rapid spread of the virus, especially among humans. Within the focus of this study, one is able to identify what has been done concerning A (H1N1) while using CDC rRT-PCR procedures. This study is also significant to individuals who would like to focus on conducting more experiments on A (H1N1). This is because the study will act as a foundation or basis for these individuals to lay out their experiments. This includes the provision of information where future experimenters may obtain their materials for conducting the experiment.

The results obtained from this experiment have an implication to the society and Centers for Disease and Prevention. This is because this study focused on an influenza virus, which is of significant concern to Centers for Disease and Prevention, as well as the society. Moreover, the study was able to illustrate how a design and typical performance of CDC rRT-PCR may be used in the process of detecting 2009 A (H1N1) pandemic influenza virus. Finally, this study indicates that there exist gap to the full conclusion of the activity of A (H1N1). This means that there is a need for conducting more and more research on the swine influenza virus in order to comprehend its impacts to the society. As such, an individual interested in reading this paper will gain more insightful knowledge as it pertains to the influenza virus. Such knowledge on the scientific study has the impact of compelling one to focus on conducting an individual research on the same, or participating in other similar research works

I believe in the data, which was obtained in this study. This is because the experiment that was performed was based on scientific facts and all the conditions for the processes, which were involved in the experiment, were followed effectively. Moreover, the spread rate of the influenza virus is rapid at a global scene. Therefore, CDC rRT-PCR analytical sensitivity of 5 copies of RNA per reaction is possible. Further, infectious doses (ID₅₀) per reaction of $10^{-1.3-0.7}$ 50% for the cultured viruses are possible. As such, the data, which is presented in the paper, has an element of reliability and credibility, which I believe that any interested person will be able to identify by reading this paper. The procedure, which was used to collect the data, is effectively explained, and the representation of the data for each procedure outlined effectively. (Q10, 17)

I really agree with the interpretations of the paper. This is because these interpretations have a scientific basis, which makes them valid for any scholar and interested individual to agree to them. Further, the interpretations are linked to the data, which was collected from the study, and they are well supported. The interpretations, which are made in this paper, provide adequate knowledge and skills to an individual on how to make accurate and valid scientific interpretations from the experiments, which are conducted. Finally, I would recommend publishing this paper as result of their convincing results (Q18).

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Authors' names:

Bo Shu, Kai-Hui Wu, Shannon Emery, Julie Villanueva, Roy Johnson, Erica Guthrie, LaShondra Berman, Christine Warnes, Nathelia Barnes, Alexander Klimov, and Stephen Lindstrom.

Performance characteristics of the Cepheid Xpert
vanA Assay for rapid identification of patients at high
risk for carriage of vancomycin-resistant Enterococci

Performance characteristics of the Cepheid Xpert vanA Assay for rapid identification of patients at high risk for carriage of vancomycin-resistant Enterococci

We learn from the title that study is about performing a rapid identification of patients at high risk for carriage of vancomycin-resistant Enterococci. It would be better if they name it “rapid identification of vancomycin-resistant Enterococci”. This study was conducted by Babady and his team at Department of Laboratory Medicine at Memorial Sloan-Kettering Cancer Center in New York (Q1, 2).

The abstract was descriptive and had information about the author's hypothesis and the experiment design. In this paper, the author used different resources to present and earlier studies to present his point of view. They compared the characteristics of direct culture and Cepheid Xpert van A in terms of sensitivity, specificity and duration of each test to identify patient with vancomycin resistant enterococci (VRE). One hundred CFU/ml was the limit of detection for Cepheid Xpert van A. One-hundred and one samples were correctly identified by this new method. This assay has a sensitivity of 100%, specificity of 96.9%, positive predictive value of 91.3%, and negative predictive value of 100%. This assay gives excellent biostatistical values when time is taken into consideration (Q3, 6)

The idea led to this research is the fact that vancomycin resistant Enterococci (VRE) are gram-positive bacteria that cause nosocomial infection, a rapid identification of this pathogen is highly important.

This pathogen causes an infection only in immunocompromised patients; however, healthy people might carry this pathogen. It is dangerous because it carries an antibiotic resistant gene which makes it almost impossible to control with antibiotics. VRE can be easily transmitted from person to person, and by eating contaminated food. A patient that has this type of pathogen must be isolated as soon as he/she gets diagnosed. There are various phenotypes of VRE, but the most prevalent phenotype in North America is vanA Enterococci. The current method of VRE identification is by culturing the sample in multiple media, incubating, gram staining and performing biochemical tests. The current method takes up to three days to get the result. In this paper, they introduce Cepheid Xpert vanA, which is a new rapid assay to identify vanA phenotype of VRE. Cepheid Xpert vanA is simply a one-step real time PCR and takes less than one hour to get the result. The paper goal is to compare this new assay to the old original culture technique in terms of specificity, sensitivity and positive and negative predictive values (Q5).

The author and his colleagues used different resources in order to present their point of view. To conduct this study, the authors used two sets of samples. They used 128 archived isolates with already known results as well as 300 consecutive rectal swabs from 162 patients. In surveillance culture, they cultured the rectal swabs onto Campy agar plates containing antibiotic discs. After that they incubated the plates at 37° with 5% CO₂ for one to two days. Then they stained suspected colonies with gram stain followed by biochemical tests to confirm the presence of VRE. In Xpert vanA PCR, they perform a PCR assay for each swab. The swabs used here were previously used in surveillance culture. In additional assay, they used vancomycin-teicoplanin Etest and enriched broth culture as a reference for discordant results. In vanA and vanB real-time PCR, primers were designed specifically for vanA gene or vanB gene to confirm

the presence of vanA gene or vanB gene. These two assays were used for samples with Xpert vanA PCR-positive, culture-negative samples. The sensitivity and the specificity of these assays were measured using Blast Local Alignment Search Tool (BLAST). In discordant result analysis, any specimen with two different results was further confirmed by either reviewing medical record or/and by culturing the specimen in enriched broth culture. In statistical analysis, the specificity, sensitivity, positive predictive value and negative predictive value were calculated for culture and Xpert vanA PCR.

To determine the lower limit of detection of Xpert vanA PCR, they used serial dilution of vancomycin resistant isolates. They tested five VRE isolates with concentration of 10 CFU/ml by the new assay and they found that only two isolates were positive; however, when they tested 5 VRE isolate with the concentration of 100 CFU/ml, the assay was able to detect all of them as positive isolates for vanA gene. This means that the limit of detection of this new assay is 100 CFU/ml.

The authors subjected 128 isolates with previously known results to the new assay. Out of 101 VRE isolates, the assay was able to detect 99 isolates as positive VRE samples using the new assay. Further tests for the two negative samples revealed that these two samples carried the vanB gene but not the vanA gene. Twenty-seven archived vancomycin susceptible samples or non-enterococci were tested using the new assay, all of them gave negative results (Table 1).

The author also used 300 consecutive samples from 126 patients. They tested these samples using Xpert vanA PCR and original culture methods at the same time. As mentioned in

the methods, the authors used reference tests on the same samples to determine false positive and false negative results. When the reference test is positive they got 56 positive samples and 18 negative samples using direct culture, and 74 positive sample using Xpert vanA PCR. However, when the reference test is negative, they got 7 positive results with Xpert vanA PCR. These results are due to the low sensitivity of culture method (75.7%) and relatively low specificity of Xpert vanA PCR (96.9%). The positive predictive value was 100% for direct culture and 91.3% for Xpert vanA while the negative predictive value is 92.6% for direct culture and 100% for vanA. In other words, samples with negative results of Xpert vanA PCR are all truly negative; however, positive Xpert vanA PCR needs further investigations (Table 2).

Out of the 300 consecutive samples, 25 samples were Xpert vanA positive/culture negative. Further investigations of these 25 samples showed that 13 samples were positive when incubated for 3 weeks in culture. 11 samples out of the remaining 12 samples gave positive results using real time PCR in another lab. To explain why they got Xpert vanA positive/culture negative they calculated the median CT value for these samples and it was 34. They performed real time PCR for samples containing different number of colonies. One sample contained only one colony and the CT value of this sample was 31 (fig1). This result explains again, that the sensitivity of the Xpert vanA assay is much higher than the sensitivity of VRE direct culture (Q7-15)

The author was successfully discussed their convincing result; they mentioned that Xpert vanA assay is a rapid diagnostic tool for VRE. This rapid identification of VRE and isolation of the patient will reduce the number of infections and outbreaks in the hospital. However, this

study has some limitations. Firstly, Xpert vanA was performed using swabs that had been used in culture. This could lower the amount of bacteria in the swab and decrease the number of false positive Xpert vanAs. Secondly, this assay detects only vanA gene but not vanB gene, which might give false negative results. Moreover, using this assay in the lab doesn't have the advantage of keeping the isolates for future studies. Someone could argue that this assay is very expensive and not worth trying. My opinion is that it is expensive in the short time; however, in the long term it is money saving because if you reduce the number of outbreaks, you will reduce the number patients and medications given. I think it will be great idea if they include vanB identification in this assay. This assay has been used in many publications; however, this paper gives the best results in term of specificity, sensitivity and positive and negative predictive value, which was interesting about this study (Table 3). Therefore, I would recommend publishing this paper, if I were a reviewer for this journal (Q4, 16, 18).

Reference:

Babady, N. Esther, et al. "Performance characteristics of the Cepheid Xpert vanA assay for rapid identification of patients at high risk for carriage of vancomycin-resistant Enterococci." *Journal of clinical microbiology* 50.11 (2012): 3659-3663.

Author's names:

N. Esther Babady, Kathleen Gilhuley, Diane Cianciminio-Bordelon, and Yi-Wei Tanga.

Genetic diagnosis of autosomal dominant polycystic kidney disease by targeted capture and next-generation sequencing: Utility and limitations

Genetic diagnosis of autosomal dominant polycystic kidney disease by targeted capture and next-generation sequencing: Utility and limitations

We can learn from the title that study is about diagnosis of autosomal dominant polycystic kidney disease using targeted capture and next-generation sequencing. The study was performed by Xiao-Ping Qi and his colleagues at University School of Medicine, Hangzhou, Zhejiang Province in China (Q1, 2).

The abstract was well written and had the author's hypothesis and the experimental design of the study. In their paper, the authors attempt to accomplish molecular diagnosis of autosomal dominant polycystic kidney disease (ADPKD) by whole-exome sequencing (WES) and targeted gene & next-generation sequencing (targeted DNA-HiSeq) of mutated Polycystic kidney disease-1 (PKD1) genes and Polycystic kidney disease-2 (PKD2) genes. The mutation in the PKD1 and PKD2 genes results in defective polycystin-1 and polycystin-2 proteins respectively. These proteins are believed to be present on kidney cells and primary cilia of renal tubules, to interact with other proteins and carbohydrates to initiate the signal that directs cells for its proliferation, movement and interaction with other cells. However, in presence of faulty polycystin-1 or polycystin-2 proteins, the normal signaling would be disrupted and gives rise to the abnormal cysts formation in the kidney. These abnormal cysts result in the ADPKD. ADPKD is the genetic, multisystem disorder characterized by cysts formation in kidney, liver, pancreas, arachnoid membranes and seminal vesicles. These facts about autosomal dominant polycystic

kidney disease were the main reason led Xiao-Ping Qi and his colleagues to do this study using different resources and information from earlier studies. (Q3-6)

The diagnosis of this disease is usually done by renal imaging, computed tomography and magnetic resonance imaging (MRI). These diagnosis techniques are highly consistent for older individuals, but less reliable in young adults and people with a disease-negative family history and produces only doubtful imaging data. To confirm the doubtful data, Xiao-Ping Qi and his colleagues aim to achieve molecular diagnosis of the ADPKD using the WES and targeted DNA-HiSeq of the PKD1 and PKD2 genes. The molecular diagnosis is also significant to identify early onset of the disease in individuals and to evaluate potential kidney donors. However, the genetic analysis of the ADPKD is extremely difficult due to the presence of large, multiple exons PKD1 and large PKD2 genes. To overcome difficulty, authors attempt to utilize the WES and targeted DNA-HiSeq techniques in the genetic analysis of the disease.

For the genetic analysis of the PKD1 and PKD2 genes using the WES and targeted DNA-HiSeq, the researchers collected DNA samples from each of two ADPKD family members (FAII-1 and FAII-2), which I would like to know according which category they selected them. At which age? And are they living in a healthy environment? However, from both the WES and targeted DNA-HiSeq sequencing, the researchers obtained million base sequences of the two genes, per patients and then they compared those base sequences with normal human genome. From the comparison, they came to know that 71% of base sequences of the PKD1 and PKD2 genes of affected patients are mutated. This suggests that mutated region can be studied further to identify potential DNA variants and can develop therapies to transform dreadful situation of

the disease. The Illumina His-Seq2000 analyzer was used to identify gene variants per sequencing lane using the sample bar-coding method in different patients.

WES disclosed 6 variants in PKD1, PKD2, and other genes associated with ADPKD e.g. DKK3 (Tables 1 and 2). The true positives in the PKD1 and PKD2 were 28.6% in the duplicated region, 75.0% in the unduplicated region, and 45.4% in the entire coding regions (Table 3). Meanwhile, targeted DNA-HiSeq disclosed 34 variants in PKD1 and PKD2. The true positives were 50% in the duplicated region, 100% in the unduplicated region, and 69.4% in the entire coding region (Table 5). Two pathogenic mutations were revealed (c.11364delC in PKD1 and c.401_401insG in PKD2) using analysis of databases and web-software and 5 variants (missense substitutions) were ruled out (Table 7). In general, there were many tables in this study which probably mislead the reader. (Q7, 8)

In Figure 1, the author describes the families that participate in this study by using a map of the both families. The diagnosis of ADPKD was based on a clinical history consistent with ADPKD and renal ultrasound and/or CT/MRI findings consistent with ADPKD. Two unrelated families with typical ADPKD and 100 unrelated healthy matched controls were included. (Q9)

In FAII-1, both the WES and targeted DNA-HiSeq effectively identified two gene variants: a heterogeneous small deletion, c.11367delC and a synonymous substitution, c.C2655T in the PKD1. In FAII-2, the WES identified three variants in the PKD1 gene; of which one appeared to be false positive and another variant in the PKD2, which can be seen in Figure 2. The WES failed to recognize a small insertion, c.401_402insG of the PKD2, which was

identified by the targeted DNA-HiSeq. Additionally, the WES identified several variants of gene DKK3, PTGS2 and RHEB, which appeared to be associated with the ADPKD. The researchers stated that the c.11367delC in the PKD1 and c.401_402insG in the PKD2 are pathogenic mutations that creates new reading frame downstream of the mutational site. There is no any experiment in this research paper to prove the researchers conclusion that the c.11367delC in the PKD1 and c.401_402insG in the PKD2 are pathogenic mutations. In future, the researchers need to work or show the details to prove this fact.

Further, to determine accuracy of the WES and targeted DNA-HiSeq techniques, the authors used Sanger DNA sequencing. In this process, the researchers compared the gene variants identified by the WES and targeted DNA-HiSeq techniques with Sanger sequencing, to determine the true positive gene variants. The WES totally revealed six variants, but Sanger sequencing confirmed only five as true positive gene variants because WES poorly identified the variants in duplicated region of the PKD1 and PKD2 genes. This result suggests that the WES might not be a good option to screen duplicated regions, but the WES is good at screening unduplicated and entire coding regions of the genes. The targeted DNA-HiSeq identified a total of thirty-four variants in duplicated, unduplicated and entire coding region of the genes. Most of the targeted DNA-HiSeq variants were confirmed as true positive variants by Sanger sequencing. This suggests that the targeted DNA-HiSeq must be more accurate than the WES, which was interesting about this study. Furthermore, the targeted DNA-HiSeq is efficient in the identifying variants and substituted variants, which cannot be identified by the WES (Q10-14).

The reason for the limitation of the whole-exome sequencing (WES) remains unanswered in this research paper. The researchers have stated that the WES did not identify some of gene variants of the PKD 1 & 2 genes in ADPKD and the WES recognized false-positive variants, but the researchers did not mention the reason for this. The perfect model to find the reason for the WES limitation would be the use of the WES to sequence any recessive disease. If the WES can recognize all potential gene variants without any false-positive, then it will be proved that the WES failed to recognize gene variants in dominant disease such as ADPKD because genes in dominant disorders have more than one rare non-synonymous variant. In general, this study shows convincing data to support the idea of using new molecular technique to diagnose ADPKD. However, including more subjects (more than 2 families with ADPKD) in the study will increase accuracy and reliability of the results. Furthermore, an experiment should be done to confirm the pathology of the two mutations in PKD1 and PKD2 because the confirmation was done only using computer software. Therefore, It would be better if the author support his study with these suggestions; however, I would accept this paper if I were a reviewer for this journal (Q4, 17, 18).

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