Leukemia Research Paper

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Background

The article that I am evaluating is studying the effects of different drugs on acute myeloid leukemia (AML). I primarily find the information on the cell cycle the most interesting in this article. Obviously, this study is not directed towards the genetic factors that cause disruptions in the cell cycle (in this case leading to leukemia), but to be able to understand how changes in your genes can disrupt the cell cycle you must first understand the cell cycle itself. While looking at this study I now know that there are many different possible steps/molecules that can ultimately be affected by mutations leading to increased risk of cancer. The purpose of this study is to identify possible drugs that may reduce AML cells or potentially cure it.

Acute myeloid leukemia (AML) is a cancer of the blood (hematologic) that originates in the bone marrow (2018; Minzel et al., 2018). This study looks at multiple different regulatory processes of the cell cycle including: CK1α, β-catenin, p53, CDK7, CDK9, γH2AX, and caspase 3. Under normal conditions, damaged DNA stimulates the production of γH2AX by phosphorylation of H2AX (Fragkos et al., 2009) which then stimulates p53. p53 is necessary for the G_1/S phase checkpoint in the cell cycle by recognizing γH2AX (result of damaged DNA) and DNA damage causing the cell cycle to halt (2017). If repair is not possible then the cell will undergo apoptosis using an intrinsic pathway. This apoptotic pathway leads to the activation of an enzyme called caspase 3 which degrades the DNA. (Steel, 2018). The p53 molecule is activated by CK1α by phosphorylation then follows the intrinsic apoptotic pathway. Another function of CK1α is to phosphorylate β-catenin, which is a type of transcription factor and in high levels promotes proliferation. The phosphorylation of β-catenin by CK1α leads to its' activation which causes degradation. The degradation of β-catenin will halt the progression of the cell cycle. (Schittek and Sinnberg, 2014; Steel, 2018). The cyclin dependent kinases 7 and 9 act as transcription factors. Specifically, CDK7 is a transcription factor for initiation and CDK9 is a transcription factor for elongation (Minzel et al., 2018). Under cancerous conditions the rate of proliferation is greater than the rate of apoptosis due to either a lack of checkpoints (p53) or an increase in transcription factors (CDK7, CDK9, β-catenin).

Process

The researchers in this study conducted numerous tests on inhibiting drugs. To identify which drug had the most effect on the AML affected cells they conducted tests such as dissociation constants (K_d), western blots, distribution graphs, Annexin V staining, tissue comparisons, and even survival curves (Minzel et al., 2018). Dissociation constants are used to evaluate the binding affinity, in this case, of the specific drugs to molecules. This will give a general idea to what drug will work best (although this is not guaranteed). Western blots are a measure of the presence and quantity of a protein. Depending on the drug used, a specific protein may be kept from being produced leading to no bands on the western blot or a protein may be enhanced leading to darker and wider bands. Distribution graphs are used to show the effect of a gene being deactivated on the number of cells that show leukemia. Annexin V is a protein that is expressed on the cell membrane intracellularly. Because of this, cells that undergo apoptosis will "flip" their cell membrane inside out. This is the first indication of cells that apoptotic. Researchers were able to identify the affect certain drugs had on AML cells by the levels of Annexin V present. Once they were able to identify which drugs to use and their general effect on the p53 pathway, they continued with

comparing the tissues of mice after being treated with the selected drugs. Finally, they compared the results of the drugs tested by compiling survival curves of the mice. (Steel, 2018).

Discussion

Figure 1A shows the results of the distribution of AML cells of a control (no genes deactivated), CK1αKO (CK1α gene deactivated), and p53/CK1αKO (p53 and CK1α gene deactivated). The graph demonstrates high levels of AML cells in the control which is expected with no treatment. When the CK1α gene is deactivated the amount of AML cells is decreased significantly. However, when both the CK1α and p53 genes are deactivated there is only a slight decrease in AML cells. This attests that p53 is necessary to remove AML cells, but only efficiently with decreased CK1α. Figure 1C expresses the dissociation constants (K_d values) of the leukemia drugs (A14, A47, A51, A64, A75, A86) with CKI, CK2, and GSK3. (Minzel et al., 2018). While only considering the K_d values for CK1α we can list the drugs from strongest to weakest binding affinity: A14, A75, A51, A47, A86, and A64. We would assume from this that A14 would have the greatest effect; however, if you analyze Figure1F which depicts western blots of these drugs it shows that all effect the levels of p53, β-catenin, and γH2AX. The two drugs that stimulate these proteins the most are A51 and A86.

Figure 2A is a graph of staining for Annexin V. As mentioned previously, Annexin V is a primary indicator for apoptosis. Because of this the levels of Annexin V can indicate the success of a specific drug. The top two drugs regarding high levels of Annexin V are A86 and A51. Figure 2B shows the resulting levels of AML cells with A51 treatment. What is shown is an obvious decrease in AML cells with A15 treatment. To further confirm the success of A51, Figure 2C examines the effect it has on each molecule in the p53 pathway. The control (with leukemia) shows no expression of p53, caspase 3, or γH2AX. These molecules are expressed in large amounts when exposed to A51 indicating that it increases the amount of apoptosis. Figure 2D is, also, expressing the success of A51 by showing the anatomical difference between the spleen and bone marrow of a control (with leukemia) and a mouse treated with A51. The spleen of the treated individual is reduced in size and the bone marrow has much more red color in it due to the proper production of blood cells. This concept is further expressed in Figure 2F by showing the histology of bone marrow, spleen, liver, and blood smear of a control and an individual treated with A51. (Minzel et al., 2018) All these tissues experience dramatic repair in response to the A51 treatment. This data is shown graphically by Figure 2E which is a distribution graph showing the levels of leukemia cells after being treated with A51. Overall, the levels of leukemia cells drop significantly once individuals have been treated with A51.

Figure 3D, E, and G are all survival curves. Figure 3D specifically looks at the survival rate of mice with leukemia without treatment and mice with leukemia with A51 treatment (Minzel et al., 2018). The results of this demonstrate that all mice without treatment eventually succumbed to the disease; however, those treated with A51 had an increased survival rate. Some still perished even with treatment, but approximately half of the individuals survived. This result was observed for A14 in Figure 3E. Figure 3G examines the results of bone marrow transplants. Healthy mice received bone marrow transplants from either an individual with leukemia or an individual that underwent treatment of A51. (Minzel et al., 2018). Mice that received bone marrow transplants from individuals with leukemia developed the disease and perished. However, the mice that received bone marrow transplants from individuals treated with A51 all survived and never developed leukemia.

The last figure, Figure 4, looks at how the different drugs being observed affect CDK7 and 9. Figure 4D shows the dissociation constants between the various drugs tested and CDK7 and 9. (Minzel et al., 2018). The order of drugs from strongest binding affinity to weakest for CDK7 is as follows: A86,

A51, A14, A47, and A64. The order of drugs from strongest binding affinity to weakest for CDK9 is as follows: A51, A86, A14, A47, and A64. Ultimately, based on K_d values the two drugs that bind the best to CDK7 and 9 are A51 and A86. Figure 4E is a western blot of drugs A51, A86, and A64. This western blot included the proteins in the p53 pathway along with CDK7 and 9. (Minzel et al., 2018). This demonstrated that A51 and A86 inhibited CDK7 and 9 while enhancing p53, caspase 3, and γH2AX.

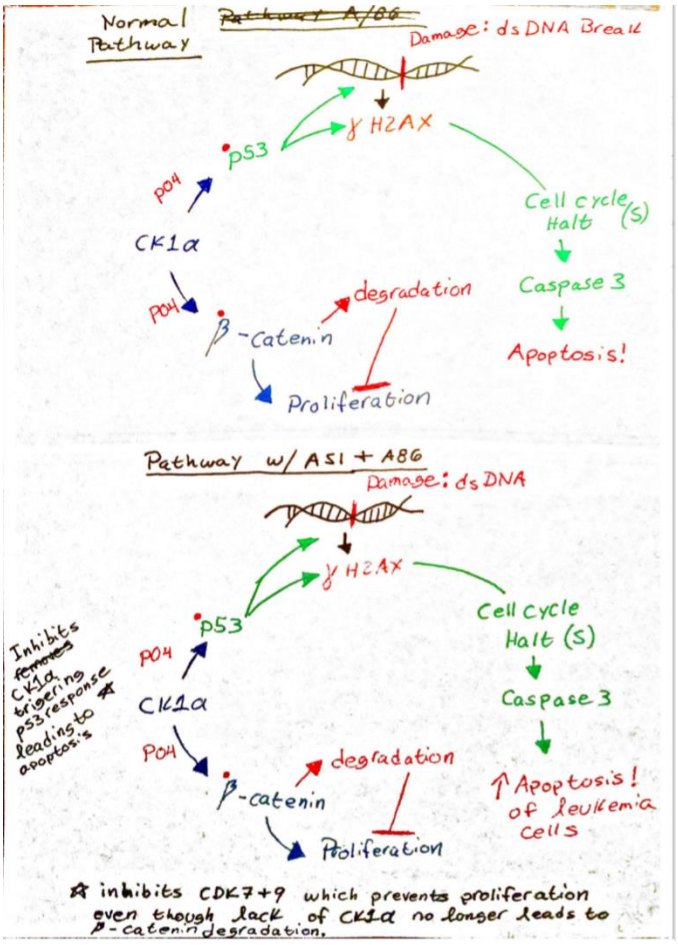

Figure a: p53 Pathway Normal vs. A51 and A86 (Steel, 2018)

Based on the results of this study, the two inhibitors that show the most promise are A51 and A86. Both drugs inhibit transcription, via CDK7 and 9, along with inhibiting CK1α which stimulates the p53 pathway eventually leading to apoptosis. However, if these drugs only inhibited CK1α then they have the potential to stimulate leukemia since transcription would increase due to high levels of β-catenin. Based on the results recorded in Figure 2, A51 and A86 lead to high levels of apoptosis in AML cells, and Figure 4 clearly shows that both drugs inhibit CDK7 and 9. By looking at the western blots of these drugs and the Annexin V stains, we can assume that A51 and A86 will cause increase apoptosis and decreased proliferation. Researchers do indeed observe these results and are recorded in Figure 2 (specifically 2B, D, and F) while observing the anatomical changes. I have attached a diagram (Figure a) showing the p53 pathway and how A51 and A86 affect it. I have, also, noted the effect of these drugs on the CDK7 and 9 pathways.

Conclusion

The main goal of the research was to develop a drug to treat and possibly cure acute myeloid leukemia. Ultimately, they were successful in isolating a drug to decrease the amount of AML cells and allow for tissue repair. I am hesitant to state that they successfully cured AML since these drugs may not work successfully in humans. Also, for someone to be cured their body must function as if they never had the disease to begin with. Since this is a type of aggressive leukemia there will, most likely, be irreparable damage. Because of this, there are some diseases that will never be truly cured. Eventually, this drug will need to undergo human trials, but I do not think we are prepared for that yet. These drugs may work in mice but could have a different reaction in humans. Before human trials can be attempted there should be human-like trials such as on chimpanzees. Only then would I recommend human trials and bringing us one step closer to “curing” the disease.

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