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The effects of gold nanorods on the rate of apoptosis of triple negative breast cancer cells

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An Honors Thesis submitted in partial fulfillment of the requirements for Honors in *Biology*

By
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ABSTRACT

Triple negative breast cancer (TNBC) is a subtype of breast cancer that is most often found in African American females that is characterized by the lack of the progesterone receptor (PR), the estrogen receptor (ER), and the human epithelial growth factor receptor two (HER2).TNBC is a very aggressive form of breast cancer because it does not respond to hormone therapy, due to the lack of the three vital receptors. Since the current treatment is not affective, the project used porphyrin to specifically target cancer in the body because it has an increased affinity for many cancer types. Gold nanorods were used as a way to quickly heat the cells upon irradiation to kill the cells with heat. The combination of the porphyrin and gold nanorods was thought to selectively target the MDA cells and trigger apoptosis due to hyperthermic ablation once exposed to IR irradiation.

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Objective

The objective of this study was to find a localized cancer treatment for triple negative breast cancer that does not induce harm on the remaining, noncancerous parts of the body. The specific aims of this study were (1) to identify whether gold nanorods themselves have a negative impact on the apoptosis rate on triple negative breast cancer cells after irradiation, (2) to determine the ideal concentration of gold nanorods to obtain the highest level of apoptosis in triple negative breast cancer cells, and (3) to determine the ideal time of irradiation to trigger the highest level of apoptosis.

Background

According to the National Cancer Institute, breast cancer is defined as a cancer that is found in the tissues of the breasts in men and women: tumors are primarily located in the ducts and lobules. Although breast cancer can occur in both men and women, it is much more commonly found in women. Between the years 2005 and 2009, breast cancer affected on average 122 women per 100,000 women in the United States for all races, making it the leading cancer incidence in women for those years.[1] There are many different types of breast cancer, and they can be classified by examining four different characteristics: where they originate from in the breast, the stage of progression of the cancer, the presence or absence of the PR, ER and HER2 protein receptors, and their morphology. [2]

MDA-MB-231 is a breast cancer reprises the loss of the progesterone receptor (PR), the estrogen receptor (ER), and the human epithelial growth factor receptor two (HER2), as found in the triple negative breast cancer (TNBC).[3] TNBC is much more

difficult to treat than many other types of breast cancer because of the lack of the PR, ER, and HER2 protein receptors. Since the current cancer therapies target these receptors, it decreases the survival rate in patients with TNBC. TNBC is responsible for 10-15% of all breast cancer cases in women.[4] TNBC and basal-like tumors are very similar in their demographics. For example, both of the cancers commonly manifest themselves in women under the age of 50, and they are more common in African American women than any other race. They are also very aggressive types of breast cancer that have approximately a 77% survival rate as compared to the 93% survival rate for many other breast cancers over a five year period.[5] New therapies are needed to treat TNBC effectively.

Nanotechnology is a scientific research field that utilizes the characteristics, behaviors and structures of nanomaterials, particles that are between one and one hundred nanometers in size. Nanomaterials generally differ from the element that they are derived from. For example, gold nanoshells do not have the same physical properties as gold does.[6] Nanomaterials currently have many research and medical applications. For example, nanomaterials are used by drug industries in order to attach different ligands that transform the nanomaterials into biosensors. One key property of nanomaterials is that they can be easily conjugated with ligands, including those that have the potential to distinguish between a diseased and normal cell.[6] This characteristic is important because of its use in targeting cancerous cells without harming the normal cells in the area, significantly reducing side effects of current chemotherapies. By changing the morphology of the nanomaterials from spheres to rods, the absorption spectrum will be changed from its original wavelength of visible light to a new wavelength of near infrared

which results in the wavelength infiltrating the human tissues much deeper than before. Moreover, Jui-Teng et al and other demonstrate the use of gold nanorods in selective photothermal therapy.[7]

Porphyrins are macrocyclic molecules that are composed of several benzyl rings covalently linked. Porphyrins are of particular interest for new cancer therapies because of their potential selectivity for cancer cells.[8] Porphyrin-conjugated compound have increased opportunities as agents for new cancer therapies because they absorb strongly in the visible light spectrum, are non-toxic to cells, are stable complexes with *in vivo* cancer affinity and are fluorescent. Porphyrins are suspected to have increased affinity for cancer cells because the porphyrins have a tendency to aggregate in cancerous tissues[8]. Additionally, they can enter the tumor through the cell membrane or endocytosis. Because porphyrin has a high affinity for serum proteins, they can gain access into vasculature, which is altered and increased in tumors. Possibly making porphyrin selective to cancer cells[8]. Combined with the gold nanorods, the porphyrin-conjugated system can potentially target the breast cancer cells and destroy them based on the gold nanorods' ability to quickly heat up the cells.

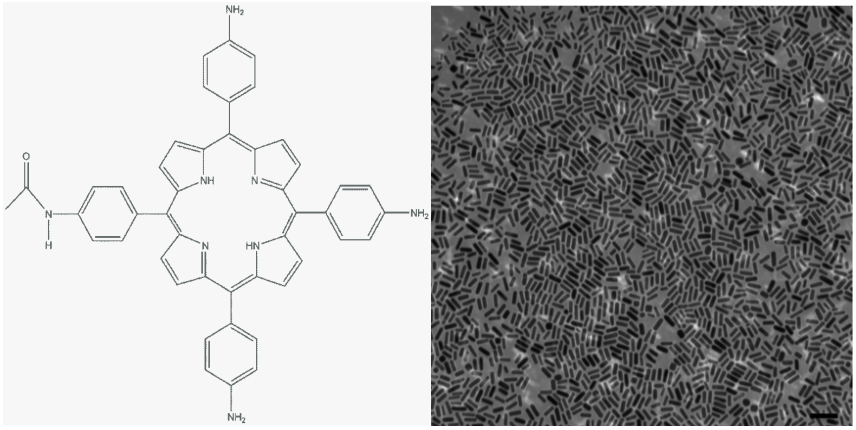

Figure 1: (Left) The porphyrin conjugated ring system that will attach to the gold nanorods. (Right) The gold nanorods that are conjugated to the porphyrin ring system in order to attach to the MDA cells.

Research Plan

Methods. The process of irradiating MDA-MB-231 cells with conjugated and unconjugated gold nanorods is a four-day procedure.

Cell culture

The experiment was conducted using MDA-MB-231 cells. They were grown in a monolayer using L-15 growth media supplemented with 10% FBS, penicillin and streptomycin. They were maintained without carbon dioxide at 37° C.

Radiation

The MDA-MB-231 cells were planted on 96-well plates at a concentration of $12x10^4$. Four plates were made: one unconjugated gold nanorods with cells without radiation, one unconjugated gold nanorods with cells with radiation, one conjugated gold nanorods with cells without radiation, and one conjugated gold nanorods with cells with radiation. The gold nanorods were added at varying concentrations of 12 µL, 14 µL, 16 µL and 18 µL, and they were placed in the incubator for one hour. After incubation, the wells were washed with PBS. The wells containing MDA-MB-231 cells are then irradiated for two minutes per well.

MTS Assay

The MTS assay was added to the wells with MDA-MB-231 cells in all four of the plates. The four plates were then placed one at a time in the spectrophotometer at 37 □C. If the average reading for each plate is not 1.2 OD, the plates must continue to be incubated until the reading is 1.2 OD.

Implications

The effects of gold nanorods on triple negative breast cancer cells have significance towards the development of new treatments for TNBC patients. A localized treatment option for aggressive forms of breast cancer could benefit the patients by targeting the specific area with cancer and not harming the noncancerous parts of the body, reducing side effects. N techniques for the treatment of triple negative breast cancer is important in counteracting the high death rate of this type of breast cancer that is largely caused by the ineffective treatment with hormonal drugs or monoclonal antibodies.[1]

Results

The MDA-MB-231 cells were irradiated using a near infrared laser at 785 nm for varying times and nanorod concentrations. The project started with a power trail to see the optimal decrease in cell viability. Figure 2 shows the percent of cell viability after irradiation for 8 minutes at varying powers ranging from 140 to 200 mW. It was determined that 140mW yielded the greatest amount of cell death after 8 minutes.

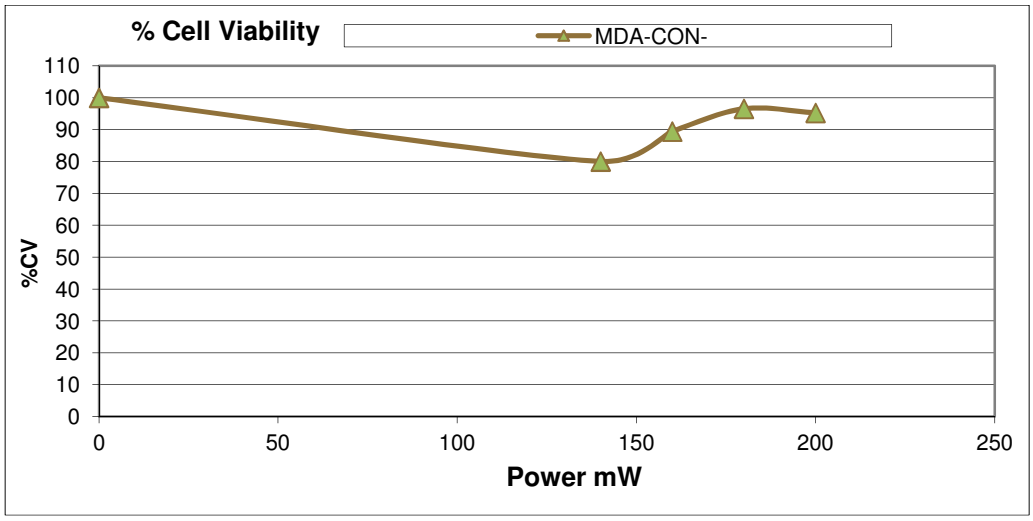

Figure 2: The percent of cell viability of the MDA-MB-231 cells irradiated for 8 minutes at varying powers ranging from 140 to 200 mW.

After the power that had the greatest cell death was determined. The MDA-MB-231, trials were done to compare the percent of cell death of MDA-MB-231 cells with and without radiation. It was hypothesized that the porphyrin would target the MDA-MB-231 cells and that the gold nanorods would yield a greater amount of concentration heat to the cells in order to trigger cell death. Figure 3 demonstrates the similarity between the conjugated MDA-MB-231 cells that have been irradiated and those that have not been exposed to radiation. The graph illustrates that cell death was not triggered in either the conjugated MDA-MB-231 cells with radiation or those without radiation. The conjugated MDA-MB-231 cells that were exposed to radiation should have shown increased cell death because of the gold nanorods concentration the heat on the cells. Since the graph illustrates that there is not a significant difference between the two conditions, it could signify that the gold nanorods were not attached to the conjugated MDA-MB-231 cells. Figure 3 was compared with Figure 4 to see if there was a difference in cell viability between the conjugated MDA-MB-231 cells and the unconjugated MDA-MB-231 cells.

The conjugated MDA-MB-231 cells were exposed to porphyrin and the unconjugated were not exposed to porphyrin. In theory, the conjugated MDA-MB-231 cells should have had more cell death because the porphyrin should have shown affinity for the MDA-MB-231 cells. However the graphs showed that there was 0% cell death in both the conjugated and unconjugated cells,

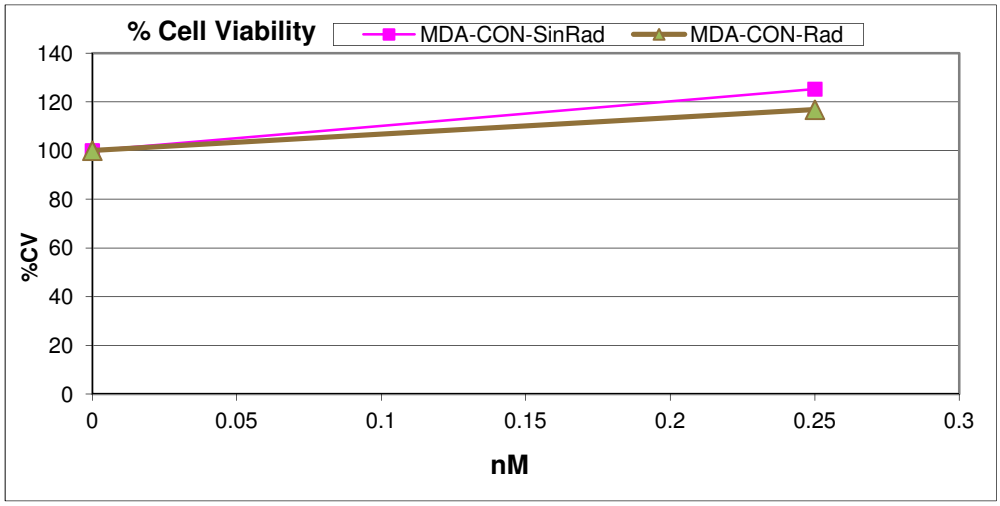

Figure 3: MDA-MB-231 cells. Cell viability after treatment with conjugated gold nanorods with and without radiation.

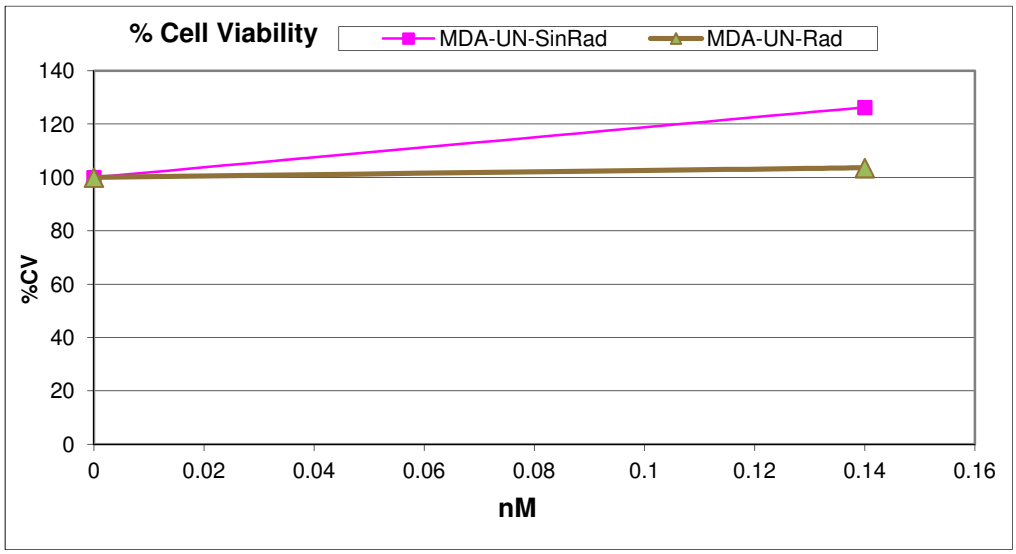

Figure 4: MDA-MB-231 cells. Cell viability after treatment with unconjugated gold nanorods with and without radiation.

The optimal concentration of gold nanorods was then determined by varying the concentrations of conjugated and unconjugated nanorods in MDA-MB-231 cells. The concentrations of the unconjuated nanorods ranged from 0.00945 nM to 0.01431 nM. While the concentrations of conjugated nanorods varied from 0.0096 nM to 0.0144 nM. Although this is a slight difference between the concentrations of nanorods between the conjugated and the unconjugated MDA-MB-231 cells, it should not have been significant enough to drastically change the percent of cell viability. Both of the graphs showed that the greatest amount of apoptosis occurred around 0.01134 nM in unconjugated MDA-MB-231 cells to 0.0112 nM in conjugated MDA-MB-231 cells that were irradiated.

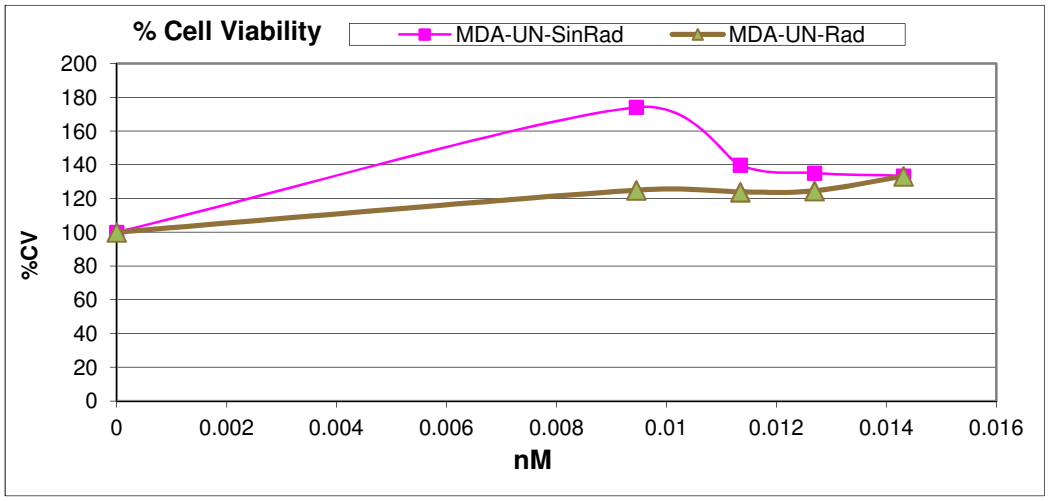

Figure 5: MDA-MB-231 cells. Cell viability at varying concentrations of unconjugated nanorods.

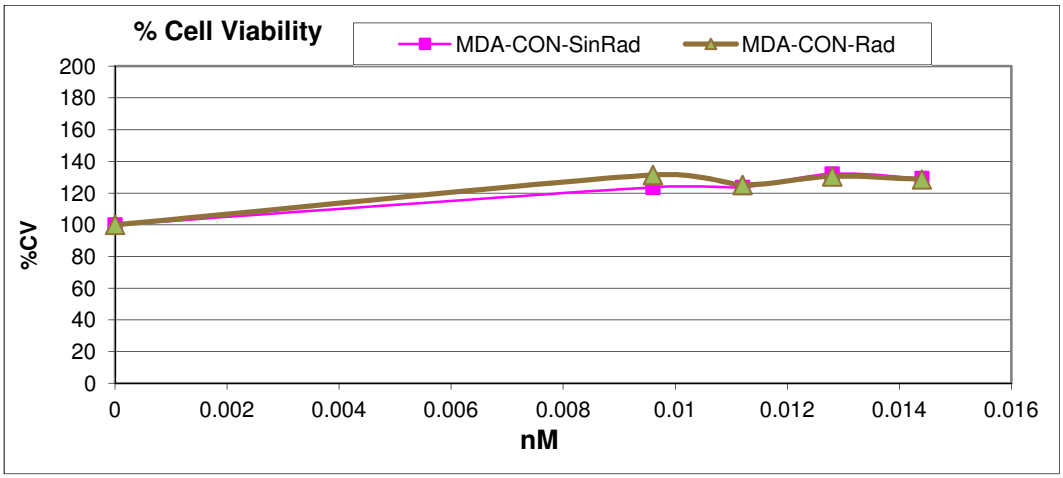

Figure 6: MDA-MB-231 cells. Cell viability at varying concentrations of conjugated nanorods.

Discussion

At the start of the experiment, a power trail was done in order to find the power that yielded the largest percentage of cell death. The trail concluded that for MDA-MB-231 cells, the optimal power was 140 mW. Trials were done with MDA-MB-231 cells at varying concentrations of gold nanorods to find the concentration that caused the most cell death. The trails were done with conjugated and unconjugated gold nanorods. Two plates were exposed to radiation and the other two were not irradiated. The results from the conjugated nanorods showed that a concentration of 0.0112 nM killed the most MDA-MB-231 cells. A concentration of 0.01134 nM triggered the most cell death in the unconjugated nanorods. The results of the trials were then compared to determine if the porphyrin was able to bind to the MDA-MB-231 cells for a specific treatment.

The project was not successfully completed in the allotted time due to many reasons. The first probable reason for the failure of the project was the inappropriate laser capacity in the Chemistry Department. The project relied on the laser being able to generate enough heat to irradiate the targeted cells by increasing the energy in the

porphyrin conjugated rods. Since the laser was not able to obtain the optimal power needed to induce to desired result, it is possible that the cells were not able to receive sufficient radiation to trigger apoptosis. Figure 2 demonstrates that varying powers have no effect on the cell viability of MDA-MB-231 cells. The most cell death at 140 mW.

Another possible reason for the failure to induce high levels of apoptosis could be attributed to the lack of affinity of the porphyrin conjugated gold nanorods for the MDA-MB-231. Figure 1 shows the structure of the porphyrin ring that is attached to the gold nanorods. The amide to the far left is the portion of the porphyrin ring system that will attach to the gold nanorod. Based on the literature, it was believed that the porphyrin rods would have an affinity for the MDA-MB-231 cells[8]; however, Figures 3 and 4 implies that the porphyrin has little to no affinity for the triple negative breast cancer cells because there was close to 100% cell viability after irradiation at 785 nm after incubation with the porphyrin nanorods. Additionally when the results from the porphyrin conjugated rods were compared to that of the unconjugated rods, there was little difference between them, as seen in Figures 3 and 4.

Both poor laser power and porphyrin's lack of affinity for MDA-MB-231 cells can be seen in Figures 5 and 6. Figure 5 shows the percentage of cell viability of MDA-MB-231 cells at varying concentrations from 0.00945 to 0.01431 nM. Figure 6 illustrates the percentage of cell viability at concentrations ranging from 0.0096 to 0.0144 nM. It was expected for the cell viability to decrease as the concentration of gold nanorods increased; however, Figures 5 and 6 showed that as the concentration increased, the cell viability increased. This either supports that the porphyrin has little to no affinity for MDA-MB-231 cells or that the power was not strong enough to induce apoptosis. It

could also suggest that the gold nanorods are not attaching to the cells, regardless of whether the porphyrin is conjugated to the nanorods or not. There is also the possibility that the rods aggregated instead of dispersing evenly between the cells. If it is necessary for uptake, that could not happen. This would mean that many of the cells were not getting the increased heat that the gold nanorods deliver, resulting in greater cell viability.

Gold nanorods are currently being used in cancer therapy research because they 1) have the ability to increase the amount of damage to the cells caused by radiation and drugs, and 2) generate heat when exposed to near-infrared radiation, which provides the possibility to increase cell death by thermal ablation [13]. Near-infrared light is the best type of radiation to use because of its ability to make a way into the deep tissues because it is has low levels of absorption by bodily fluids [14]. Gold nanorods are used because they are able to decrease the amount of light that results from interactions with surrounding tissues. Because they limit the unnecessary light interactions in the surrounding tissue areas, gold nanorods also limit the healthy tissue damage as a result of radiation [14]. It has been published that cell death will occur when the irradiated sample reaches 41°C-47°C using near-infrared light [15]. Hyperthermia results in the denaturing of the cellular proteins which weakens the cellular membrane. Its use as a cancer therapy is to inhibit tumor cell growth by means of triggering apoptosis in the tumor cells or making the cells more susceptible to radiation or chemotherapy [15].

It is possible that this project was not irradiating the MDA-MB-231 cells long enough to reach the necessary temperature to trigger apoptosis, or a more powerful laser may have been needed to reach the target temperature range. The cells were irradiated using less media in hopes of reaching a higher temperature; however, less media resulted

in the wells drying too quickly during the irradiation process. It has been suggested that the pulsed-mode laser is the more suitable choice for cancer therapies because it allows for more efficient photothermal conversion [14]. In the future the research could be improved by discovering a way to 1) measure the temperature that irradiation with nanorods is giving off compared to samples without nanorods, and 2) achieve the optimal temperature without causing greater amount of cell death by stressing the cells due to the decreased level of media. The project could also change the type of laser used to the pulsed-mode laser in hopes of delivering more efficient and stronger beam to the MDA-MB-231 cells.

Conclusion

Although this experiment did not yield the expected outcome, there is hope for the future of localized breast cancer therapies. The particular porphyrin that was chosen for use in this experiment is one type among many. In the future, some of the issues with this experiment, such as a lack of affinity for the MDA-MB-231 cells by the porphyrin could potentially be resolved by using a different type of porphyrin. The other major issue with this experiment, the lack of power, could possibly be resolved by using a stronger laser with less refraction over a longer period of time, in hopes of increasing the rate of cell death.

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References

[1] U.S. Cancer Statistics Working Group. *United States Cancer Statistics: 1999–2009 Incidence and Mortality Web-based Report*. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute; 2013. Available at: www.cdc.gov/uscs.

[2] Facts for Life: Triple Negative Breast Cancer. (2011). In *Susan G. Koman for the Cure*. Retrieved April 27, 2013, from http://ww5.komen.org/uploadedFiles/Content_Binaries/KOMEED079100.pdf

[3] Wen-Hung, K., Yao-Yin, C., Liang-Chuan, L., Mong-Hsun, T., Chuhsing Kate, H., King-Jen, C., & ... Ju-Seog, L. (2012). Molecular Characteristics and Metastasis Predictor Genes of Triple-Negative Breast Cancer: A Clinical Study of Triple-Negative Breast Carcinomas. *Plos ONE*, *7*(9), 1-11. doi:10.1371/journal.pone.0045831.

[4] C.K. Anders and L.A. Carey, Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer, *Clin Breast Cancer* **9**(Suppl 2) (2009), S73–S81

[5] Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype. Cancer. 2007; 109(9): 1721–8.

[6] Ferrari, M., Downing, G. (2005). Medical Nanotechnology. *Biodrugs*, 19(4), 203-210.

[7] Jui-Teng, L., Yueh-Sheng, S., Guang-Hong, L., & Hsia-Wei, L. (2012). In Vitro Photothermal Destruction of Cancer Cells Using Gold Nanorods and Pulsed-Train Near-Infrared Laser. *Journal of Nanomaterial,* 1-6. Doi:10.1155/2012/861385.

[8] Vicente, M. (2001). Porphyrin-based sensitizers in the detection and treatment of cancer: recent progress. *Current Medicinal Chemistry. Anti-Cancer Agents, 1(2),* 175-194.

[9] Stadalnykaité, S., & Briediené, R. (2011). Radiological diagnosis of triple negative breast cancer: a review. *Acta Medica Lituancia*, 18(2), 98-106.

[10] Eng-Wong, J., Zujeqski, J., Chavez, K.J., Garimella, S. V., & Lipkoqitz, S. (2011). "Triple negative breast cancer cell lines: One tool in the search for better treatment of triple negative breast cancer." *Breast Disease* 32, no. 1-2: 35-48.

[11] Kruse, M., Brufsky, A., % Puhalla, S. (2012). Triple-Negative Breast Cancer. *Current Medical Literature: Breast Cancer*, 24(4), 131-141.

[12] Ménars, S. S. (2012). Heterogeneity of triple-negative breast carcinomas. (English). *Omcologie*, 28-30. Doi:10.1007/s10269-001-2104-y.

[13] Jelveh, S., & Chithrani, D. B. (2011). Gold Nanostructures as a Platform for Combinational Therapy in Future Cancer Therapeutics. *Cancers*, 3(1), 1081-1110.0 doi:10.3390/cancers3011081.

[14] Choi, J., Yang, J., Jang, E., Suh, J., Huh, Y., Lee, K., & Haam, S. (2011). Gold Nanostructures as Photothermal Therapy Agent for Cancer. *Anti-Cancer Agents in Medicinal Chemistry, 11*(10), 953-964.

[15] Fernandex Cabada, T., de Pablo, C., Serrano, A., Guerrero, F., Olmedo, J., & Gomez, M. (2012). Induction of cell death in a glioblastoma line by hyperthermic therapy based on gold nanorods. *International Journal of Nanomedicine*, 71511-1523, doi: 10.2147/IJN.S28470.