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Paving Way for a Paradigm Shift in Oncology: Curing Cancer by Loving It?

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Abstract: Plateaus in the efficacy of traditional methods for the treatment of cancer reached in the last decades call for the exploration of alternative models as their potential clinical complements. Here, the classical view of cancer as a tissue that is to be eradicated by methods describable by a compendium of militaristic metaphors is being challenged with a provocative idea: what if cancer can be cured with love condensed down to the level of molecular and cell biology? Correspondingly, the idea that love mimics the traits of the objects of its affection and helps them grow was translated to the level of cell biology by incorporating anti-apoptotic properties in healthy cells and promoting tumorigenesis in cancerous cells. Both the indirect and direct co-culture of the two cell types demonstrated hindered growth of cancer cells relative to that of their primary counterparts when these cellular modifications inspired by love for cancer were being implemented. The two experimental models reported here are emphasized as crude and simplistic methods derived from the idea that cancer may be best treated by being loved at the cellular and molecular biology levels. More comprehensive and effective methods may emanate from continued exploration and expansion of the intriguing and innovative avenue for cancer management proposed here.

Keywords: autopoiesis; cancer; co-culture; E297; love; p53; RUNX2

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1. Introduction

"*Love your disease*" (J. Harrison, 1984 [1] & V. Sinelnikov, 2014 [2])

To live forever has been one of the most persistent dreams of human beings. But how ironic it is that the biological entity that is closest to teaching us the secrets of immortality is the one feared most in the modern age: the cancer cell. Quite logically, given its deadliness, cancer, traditionally, has been a thing to defeat and eliminate rather than be instructed by. Yet, what if the cancer cell is here to teach us the secrets of the infinite, of the eternal life sought by generation after generation of humans? What if it is to be embraced and empowered rather than hated and destroyed? What if it is to be "loved" if we are to heal the organisms ruptured internally across the divide between cancer cells and healthy cells? This question will be elaborated upon from the medical standpoint rooted in the fundamentals of molecular and cell biology in this brief research report.

Phrases containing clear warlike connotations, such as "battling", "targeting", "invading" "fighting", "beating", "armoring" and "destroying", are commonly used by researchers, clinicians and caregivers in oncology to denote their relationships with cancer [3]. These phrases are not only pervasive in the literature and verbal communication, but also have no alternative when it comes to envisioning the approaches to tackling cancer as a disease [4]. These violent, militaristic metaphors have been shown to lead to the feelings of disempowerment and fatalism among cancer patients [5]. On the therapeutic side of things, this traditional, mainstream way of seeing cancer as a tissue to S&D—i.e., Search and Destroy, as if medicine is warfare [6] and not a higher art—has yielded some benefits for the longevity and comfort of the patient, but steady plateaus have been reached in the last decades for many cancers, and the improvements in survival statistics and life quality

have been marginal ever since. Breast cancer [7], small-cell lung cancer [8], osteosarcoma [9] and glioblastoma [10] are some of many of the cancer types for which plateaus in survival rates have been reached. Furthermore, a longitudinal trend analysis looking at the cancer survival rates between 1971 and 2011 in Great Britain showed no improvement over time for poor-prognosis cancers, such as those of the esophagus, stomach, pancreas or brain [11]. As for the latter, with all the advances in the genetic and molecular profiling of patients, in high-precision surgery, in imaging and radiation therapy, in pharmacopeia and in targeted drug delivery, the median life of patients with glioblastoma multiforme has increased by a mere four and a half months since 110 years ago, when the first cohort of tumor resection craniotomies for the treatment of this disease was reported [12]. A portentous sign that impasses have been reached in the traditional approaches to treating cancer based on the classical triad of surgical resection, chemotherapy and radiation also comes from the unprecedented recent expansion of investment in R&D of early diagnostics as a hallmark of personalized medicine. This, however, to those who have dedicated their lives to the therapeutic aspects of tackling this deadly disease, comes forth as one way of admitting defeat against a mighty enemy.

These plateaus in survival necessitate an exploration of alternative therapeutic models, if not a radical paradigm shift in the way that medicine approaches the treatment of cancer. For one, the consistent reliance on warlike metaphors in oncology parlance should be recognized for its detrimental effect on the conception of these alternative models that may overcome the survival plateaus faced by the mainstream modes of therapy. One such alternative point of view, from which a plethora of related models could spring to life, is being proposed here. Its elaboration may start with the question whether cancer, as opposed to being perceived as a wartime enemy, is to be loved and if we should try to integrate it within the host in order for its malicious growth to stop. This may seem like a childish remark to many, but what if this would indeed be possible, and what if, with one such loving approach, the destructive energy of cancer could be exhausted and harnessed for something positive. If so, what would be the path leading to this noble and starry-eyed objective? What would comprise this "love" in the language of molecular and cell biology? This is what the current study of a rather modest empirical scope attempts to elaborate on. Given the novelty of this approach and limited resources for experimentation, it should be accepted that not definite answers but only sketches of the path leading to them can be provided here.

The first point to recall on this scientific journey is that cancer is an interfacial issue. In other words, the interface between the malign cell or a conglomerate thereof and normal cells defines the genesis and progression of cancer. This can be exemplified by the fact that mutated cells with blocked apoptotic pathways commonly form in tissues, but are also equally commonly eliminated by the immune system in their microenvironment before they can grow into tumors [13]. Over time, therefore, the idea that conduciveness of this microenvironment to tumor progression is the key factor in carcinogenesis has gradually ripened into a whole new paradigm in oncology [14]. Today, as a result, it is being widely accepted that the cellular and stromal niche surrounding the cancerous tissue is greatly responsible for the rise of resistance to therapy [15] and the overall tumor resilience [16]. Consequently, tumor stromal cells [17], fibroblasts [18], adipocytes [19], context-dependent autocrine factors [20] and paracrine loops between tumor cells and macrophages [21], alongside other immune cells [22], including T cells, B cells, dendritic cells and monocytes, can all be imagined to form a complex multicellular microenvironment whose characteristics determine the tumor progression rate. With the constant accrual of reports confirming the role of the cancer microenvironment in the tumor development, this physiological surrounding of the tumor, rather than the tumor *per se*, has started to be seen as the region where the therapeutic effect is ideally to be delivered. The major practical corollary of this new paradigm is that the treatment of cancer at the cellular scale need not target cancer cells *per se*, but can also focus on instructing their normal cellular neighbors on how to eradicate the tumor, as through vaccines [23], chimeric antigen receptor (CAR) T-cell

therapies [24] or programmed death-ligand 1 (PD-L1) checkpoint inhibitor therapies [25] among others. In general, this has solidified immunotherapy as a major new direction in the treatment of cancer.

However, it should be recognized that the new cancer immunotherapies [26], where the attention is switched from killing the cancer cell to empowering the immune system to recognize it and keep its malignancy in check, are only a softer version of the traditional "warfare" model, notwithstanding that they may act as the first step in the transition from aggression to "love" in the way anticancer therapies are conceived. This is because the idea that cancer is the enemy and a "target" rather than the object of admiration, love and affection remains intrinsic to this therapeutic model, just as it is intrinsic to the "warfare" model. Yet, it is hypothesized here that the continuation of the reversal of the paradigm that has begun with the idea that the immune system is to be empowered to annihilate the cancer tissue instead of directly and crudely attacking the latter is imperative. It is possible that only this approach may lead medicine to the accomplishment of a more desired goal, which is to therapeutically impel the cancer cells to lose their malignancy and engage in a symbiotic relationship with the healthy tissues, if not to convert back to normal cells. It is clear that only if these latter endpoints in treatment are reached can the cancer patients emerge from the point of care lastingly healed.

2. Approach

If the etiology of cancer is assumed not to be reducible to an intracellular mutation issue alone and it is accepted, instead, that this medical pathology arises from the divorce of the neoplastic tissue from its healthy microenvironment, then the only prospect for healing cancer lies in directing the major therapeutic effort at the interface between the healthy and the pathological tissues. If a divorce, in other words, is pending or ongoing, then the healing, poetically speaking, must be about the promotion of love between the tissues that have become estranged.

There are two different paths that are explored in this conceptual study in the effort to make this giant step from the old and traditional, inherently aggressive way of treating cancer to the new and "romantic" way of achieving the same. Both methods can be perceived as natural corollaries of love for the malfunctioned, cancerous tissue. The first of the two methods is based on allowing the healthy cells to take on a cancer cell feature, in analogy to the way a person in love adopts the personality traits of the object of his/her affection. This first approach can be said to have been motivated by the path taken on by Yamanaka et al., whose Nobel Prize-awarded research [27] was on converting the phenotype of differentiated cells back to a pluripotent state when the rest of the stem cell research community rushed to find means of differentiating stem cells into specific lineages [28]. Similarly, here, in times when the dominant course of action in the field of genetic engineering for cancer mitigation, the immunotherapeutic efforts notwithstanding, is still to modify the cancer cell by introducing specific therapeutic factors to it—be it by means of extracellular vesicles, small interfering RNAs (siRNAs), CRISPR/Cas9 gene editing, controlled delivery of chemotherapeutic drugs or other biomolecules to shut down the cell metabolism—the healthy cells adjacent to the cancer cells will be altered in their makeup in such a way that they begin to resemble the latter cells more, not less. In this first approach followed in this study, normal fibroblasts neighboring the cancer cells, being the essential components of the tumor microenvironment [29], are subjected to this alteration.

Counteracting the mainstream effort of interfering with the deadliest elements of the cancer cell with the purpose of shutting the cell metabolism down, here it is suggested that these elements may need to be sought, excavated, isolated and introduced to the normal cells in the tumor microenvironment. This approach can be seen as analogous to the way eukaryotic cells embraced and internally integrated the mitochondrion from their prokaryotic neighbors in the evolutionary past [30], a process which is now thought to be intimately tied with cancer etiology [31]. This endosymbiotic process may be instructive with regard to the mechanism by which a specific malignant or nonmalignant feature of

the cancer cell could be incorporated inside the healthy host cell for an evolutionarily benevolent purpose or direct patient benefit. Here, it is thought that by delineating what the analogy of the mitochondrion as the quintessential feature of the cancer cell is and mimicking the biomolecular mechanism by which the mitochondrion was incorporated into the eukaryotic cell, the cancer tissue microenvironment could be modified in a way that stops its growth and its invasion of the healthy surrounding. Out of a number of possible analogies for the mitochondrial acquisition in this context, here it is hypothesized that (1) enabling the tumor-suppressor checkpoint deregulation in healthy cells, just like the ones that are intrinsic to the cancer cell; (2) activating the immortalization mechanism by shutting down the apoptotic pathways in a health cell adjacent to the tumor; or (3) saturating the healthy cell with surface receptors, such as the epidermal growth factor receptor (EGFR) overexpressed in some cancer cells, would be able to halt the tumor growth. To test this hypothesis, a simple experiment is being chosen, involving the suppression of p53 in the post-transcriptional and pre-translational stage in normal fibroblasts co-cultured with the cancerous tissue. The given protein, p53, is a checkpoint macromolecule involved in the suppression of tumorigenesis [32], while the gene encoding for it, TP53, is considered the most frequently mutated gene in human cancer [33]. Although the restoration of normal p53 expression levels has been studied abundantly as a potential form of anticancer therapy, no such therapies are clinically available yet [34].

The second approach followed in the attempt to delve into this paradigm-shifting idea that "loving" cancer can be the route to healing the organism is based on another essential trait of love, which is to bestow upon the object of one's affection what is most beneficial for its growth and well-being. This approach, once again, counteracts the mainstream idea of modifying the cancer cell with the goal of obliterating its most toxic structural elements. Instead, it does the very opposite: it supplements the cancer cell with elements that it would love to have on its mission to live forever. Simplistically speaking, if cancer is to be stopped from progressing more effectively by being loved and nourished than by being perceived as an enemy targeted for elimination, then it should be given what it wants, and there are three things that it craves: (1) to live forever, (2) to be more invasive and (3) to multiply at ever faster rates. As for (1), although transfecting cancer cells with additional mutated genes that transcend the apoptotic checkpoints in anticipation that they would become saturated with them and undergo growth reversal is a valid idea, the apoptotic pathway in these cells is already shut, so adding another anti-apoptotic gene would be a redundant and questionably sensible approach. As for (2), making cancer more invasive would require a highly complex, multifaceted approach. Realistically, however, considering (3), cancer tissue can be made to multiply faster, hoping that, in the process, it would run across an error and autodestruct. Accordingly, in this study, the cancer cells are transfected with exogenous osteogenic master gene RUNX2 (runt-related transcription factor 2), whose DNA-binding RUNT domain has been implicated in the progression of several cancers. Cancer Genome Atlas data analyses, for example, showed that RUNX2 is a gene that is consistently overexpressed in numerous cancers, including renal, esophageal, gastric and pancreatic [35]. The deletion of the RUNT domain using the CRISPR/Cas9 technique lowered the cancer cell migration by a considerable margin [36], while its presence contributed to increased osteotropism and bone invasion in melanoma cells [37]. RUNX2 has also been shown to promote the aggressiveness of malignant gliomas [38] and act as a master transcriptional regulator in lung cancer [39] and in possibly all other cancers. RUNX2 is also a key epigenetic enhancer of tumor metastasis, stemness and resistance to therapy [40]. Last but not least, RUNX2 and p53 are antagonistic in effect, given that RUNX2 acts as a negative regulator for pro-apoptotic p53 in response to DNA damage [41] and the interaction between the two proteins through the RUNX2/p53 complex may alter the activity of histone deacetylase [42].

In short, two distinct methods evocative of love for cancer were pursued in this study: one involving the altering of the composition of normal cells of choice, namely primary fibroblasts, and the other one involving the altering of the composition of a cancer cell line of choice, namely E297 glioblastoma. Both of these approaches were accomplished through the

transfection of cells, using calcium phosphate (CaP) nanoparticles as the inorganic nonviral transfection agent. To achieve this compositional and structural alteration at the cellular level, modulation of protein expression was carried out either at the post-transcriptional stage, by employing siRNA, or at the pre-transcriptional stage, by employing plasmid DNA. siRNAs are single-stranded non-coding RNAs composed of 20–30 nucleotides, whose sequence is made complementary to a portion of the mRNA whose translation is to be prevented by slicing it. Plasmid DNA, in contrast, is a genetic sequence encoding for a particular protein that is expressed after the plasmid becomes internalized by the cell. A major challenge for the intracellular delivery of nucleic acids comes from their polyanionic nature, which is not conducive to the transport across the lipid bilayers of the cellular membrane [43]. CaP nanoparticles, however, possess an excellent uptake potential by virtually all cell types [44–46] and have been used with success for the delivery of nucleic acids, ranging from DNAs [47] to RNAs [48], including siRNA [49], and extending to various other oligonucleotides [50].

3. Materials and Methods

3.1. Synthesis of CaP Nanoparticles

The synthesis of CaP nanoparticles proceeded by adding 25 mL of 0.1 M aqueous solution of calcium nitrate ($Ca(NO_3)_2$, Fisher Scientific, Waltham, MA, USA) containing 1 mL 28% ammonia (NH_4OH, Sigma Aldrich, Burlington, MA, USA) dropwise to the same volume of 0.06 M aqueous solution of monoammonium phosphate ($NH_4H_2PO_4$, Fisher Scientific, Waltham, MA, USA) containing 0.5 mL 28 vol.% NH_4OH. After the addition of $Ca(NO_3)_2$ to the phosphate solution and vigorous stirring with a magnetic bar (400 rpm), the precipitate was centrifuged for 5 min at 3500 rpm, after which the supernatant was decanted. The precipitate was then washed with deionized water, separated from the supernatant by centrifugation and stored at 4 °C until use. To load CaP nanoparticles with nucleic acids as transfectants, either siRNA in the amount of 50 µg or plasmid DNA in the amount of 10 µg was added to the calcium solution during the precipitation of CaP. It was made certain that the pH of the reaction solution during the synthesis did not exceed 9, which was the previously tested threshold for siRNA stability [51] and binding activity [52]. The efficiency of loading CaP with the nucleic acids, A, was determined as the average of three independent measurements, using the following equation:

$$A = \frac{N_{total} - N_{supernatant}}{N_{total}} \quad (1)$$

where A is the loading efficiency, N_{total} is the total amount of the nucleic acid added to the system, and $N_{supernatant}$ is the amount of the nucleic acid left in the supernatant after the precipitation and isolation of CaP. Nanodrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to measure the concentration of nucleic acids in different media and the loading efficiency, A, was in the excess of 95% for all the samples.

3.2. Characterization of CaP Nanoparticles

High-resolution scanning transmission electron microscopy (STEM) analysis was carried out on a JEOL 2100F microscope equipped with a Schottky-type field emission source and a cryo-polepiece operating at 200 keV. All images were recorded using a Gatan OneView camera with a point-to-point resolution of 0.26 nm, lattice resolution of 0.1 nm and information limit of 0.124 nm. The phase composition of CaP nanoparticles was confirmed on a Bruker D2 Phaser X-ray diffractometer, using polychromatic Cu as the irradiation source. K_β line was stripped off with an inbuilt filter, whereas $K_{\alpha 2}$ line was stripped off manually. The step size and the sample irradiation per step were 0.01° and 1 s, respectively.

3.3. Cell Culture

E297 human glioblastoma cells were a gift from H. Engelhard (University of Illinois, Chicago, IL, USA) and were used as cancer cells in this study. Kidney fibroblasts were iso-

lated from the kidneys of 8-week-old female C57BL/6J mice and were used as the primary cells in this study. The cell isolation started off with mincing kidneys in Hanks' balanced salt solution (Life Technologies, Carlsbad, CA, USA) and digesting using collagenase type IV in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 5 % antibiotic–antimycotic (Life Technologies, Carlsbad, CA, USA). After 90 min of incubation at 37 °C with intermittent shaking, the cell debris was allowed to settle by gravity, and cell suspensions were transferred to 10 cm culture plates with 10 mL of the suspension per dish. Both cell types were grown in the abovementioned DMEM medium supplemented with 10% FBS and 1% antibiotic–antimycotic solution. Passaging was performed upon confluence, by detaching the cells from the surface of 75 cm^2 cell culture flasks (Greiner Bio-One, Monroe, NC, USA) with 0.25 wt% trypsin and resuspending the cells at a 1:7 volume ratio in 10 mL of fresh media. The medium was replaced every 48 h, and the cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The cultures were regularly examined under an optical microscope to monitor the growth and check for possible contamination.

3.4. Cell Treatment

To impart a property onto primary cells suggestive of love for cancer, the primary fibroblasts were treated with CaP nanoparticles loaded with p53 siRNA (sc-29435, 3.3 nmol, Santa Cruz Biotechnology, Dallas, TX, USA). To that end, nearly confluent primary cells cultured in 24-well plates were transfected with 5 mg/well of CaP nanoparticles loaded with siRNA and incubated overnight. In contrast, to impart a property onto cancer cells that is suggestive of love for them, trying to enhance their proliferation, E297 glioblastoma cells were treated with CaP nanoparticles loaded with the plasmid DNA encoding for human RUNX2 (OriGene Technologies, Inc., Rockville, MD, USA) and incubated under the same conditions as those reported for transfection with siRNA.

3.5. Transwell Co-Culture Assay

Pretreated primary fibroblasts were seeded at 2.5×10^4 cells/mL on transwell inserts (Corning) with a high pore density and placed inside 24-well plates simultaneously seeded with E297 cells at the same density. Both cell types were grown in 300 µL of the DMEM cell culture media supplemented with the antibiotic and antimycotic solution at 37 °C. Periodically, the cells were detached from the surface and subjected independently to a viability analysis, using the 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. The 12 mM stock solution of MTT was prepared by adding 1 mL of sterile PBS to a 5 mg vial of MTT and vortex mixing to ensure complete dissolution. This solution in the amount of 275 µL of 1:10 MTT/media vol/vol was added in each well and transwell containing cells. After 4 h of incubation at 37 °C, 200 µL of the solution was removed, and 125 µL of dimethyl sulfoxide (DMSO) was added to each well. Plates were placed in a 37 °C incubator shaker at 120 rpm for 30 min before measuring the absorbance at 570 nm, using the BMG LABTECH FLUOstar Omega microplate reader. The ratio between the average absorbance values corresponding to the two cell types in their co-culture was plotted as a measure of their comparative rates of growth and metabolic activity. All experiments were performed in quadruplicates and subjected to an unpaired t-test for a statistical difference estimation.

3.6. Spheroid Co-Culture Assay

Cancer cells and primary fibroblasts, after the treatment, were seeded at a 1:1 ratio onto 96-well low adhesion plates at 10^4 cells/mL for each cell type. Cells were incubated at 37 °C and 5 % CO_2 for 48 h to allow spheroids to form. Spheroids were then removed and placed in the wells of a 48-well plate pretreated with 0.1 wt.% gelatin for 30 min. Under these conditions, spheroids were incubated and periodically sampled out for a migration rate analysis. Prior to analysis, the spheroids were washed with PBS and fixed with 4 vol.% paraformaldehyde (PFA) for 1 h. Spheroids were then imaged under bright-

field conditions, using a Nikon T1-S/L100 inverted epifluorescent confocal microscope. Cell migration from spheroids was measured as the distance from the leading edge of the cells migrating out of the spheroid to its surface, using ImageJ (NIH, Bethesda, MD, USA). All experiments were performed in quadruplicates and subjected to an unpaired t-test for statistical difference estimation.

4. Results and Discussion

In the effort to think of a fundamental new approach to treating cancer, the idea of conceiving of treatments on the basis of loving cancer was conceptualized and experimentally tested in a most preliminary fashion. As explained in the introductory section, love is being perceived in this context as the source of two effects with respect to the interface between the cancer cells and the healthy cells in their microenvironment. One is to give the object of one's affection—in this case, the cancer cell—what it needs to grow; and the other one is for the system in love—in this case, the healthy cell—to adopt the characteristic traits of the object of its affection. Both of these effects are produced with the long-term intention of halting the growth of the neoplastic tissue and facilitating the disintegration of the pathology, along with its reabsorption by the adjacent healthy tissues. Out of innumerable possible properties and methods for imparting them, two simple ones were tested here, one involving the silencing of the tumor-formation suppressor, p53, in healthy cells, and another one involving the augmentation of the expression of RUNX2, a promoter of neoplastic aggressiveness, in cancer cells.

Two fundamentally different co-culture models were chosen for the purposes of this study, one of which is classifiable as indirect and the other one as direct [53]. The two models, the indirect and the direct, were used to measure the effects of the treatment on the cell viability and on the tumor migration rate, respectively. The indirect co-culture involved culturing primary cells on the apical side of transwells and cancer cells on the bottom of the dishes over which the transwells were suspended [54]. The direct co-culture model involved culturing spheroids comprising one cancer cell line and one type of primary cells homogeneously mixed therein [55]. To impart properties suggestive of loving cancer onto primary cells and cancer cells, transfection with siRNA and plasmid DNA, respectively, was accomplished using CaP nanoparticles as nucleic acid carriers. The as-prepared CaP nanoparticles assumed elongated, needle- and plate-shaped morphologies with a relatively narrow distribution of sizes. The width of CaP nanoparticles was in the 5–10 nm range, while their lengths were 100 nm or more, and their aspect ratios ranged between 5 and 10 [56]. As it can be seen from the STEM image reproduced in Figure 1a, however, the nanoparticles were moderately agglomerated. This natural propensity for agglomeration, in fact, presents a physical effect harnessed here to capture the nucleic acid cargo and transport it to the interior of the cell. For this reason, the nucleic acids were made to be present in the reaction system during the precipitation of the nanoparticles instead of being added as an adsorbate afterwards (see Section 3.1). In such a way, the nucleic acid molecules became tightly confined within the pores of the nanoparticle agglomerates, without being able to escape by desorption to a significant degree. The X-ray diffraction analysis confirmed that CaP nanoparticles consisted of a single phase, namely hydroxyapatite (Figure 1b), the most sparsely soluble of all CaP phases [57].

The viability effect of the two methods tested, one of which involved the suppression of p53 expression in primary fibroblasts and the other one of which involved the augmentation of the expression of RUNX2 in cancer cells, is shown in Figure 2 for the indirect co-culture model, where the two types of cells grew separate from one another but still shared media through which active signaling molecules and metabolites were being exchanged. According to the results presented in Figure 2, the growth of the cancerous component of the co-culture was suppressed relative to that of the normal cellular component of the co-culture on both days 1 and 4 for both types of cellular transformation, as compared to the untransformed control co-culture (Day 1: p53 $p = 0.5205$ vs. control, RUNX2 $p = 0.3673$ vs. control; Day 4: p53 $p = 0.4090$ vs. control, RUNX2 $p = 0.2180$ vs. control). On day 7,

the last day of the experiment, the co-culture containing cancer cells with an augmented expression of RUNX2 continued to exhibit lower levels of cancer growth relative to the control (p = 0.0572), whereas the co-culture containing primary cells with a suppressed expression of pro-apoptotic p53 diverged from this trend and exceeded the cancer growth of the control co-culture (p = 0.7559). In all cases, the cancer growth exceeded that of the normal cells due to intrinsic cell-cycle reasons, but the reduction in the growth rate normalized to the growth of the normal cells is promising. The fact that the pre-translational p53 suppression provides only a temporary effect on this comparative growth rate may suggest that this type of RNA therapy is to be delivered with a higher frequency compared to that involving RUNX2 upregulation through the transfection with considerably more stable plasmid DNA.

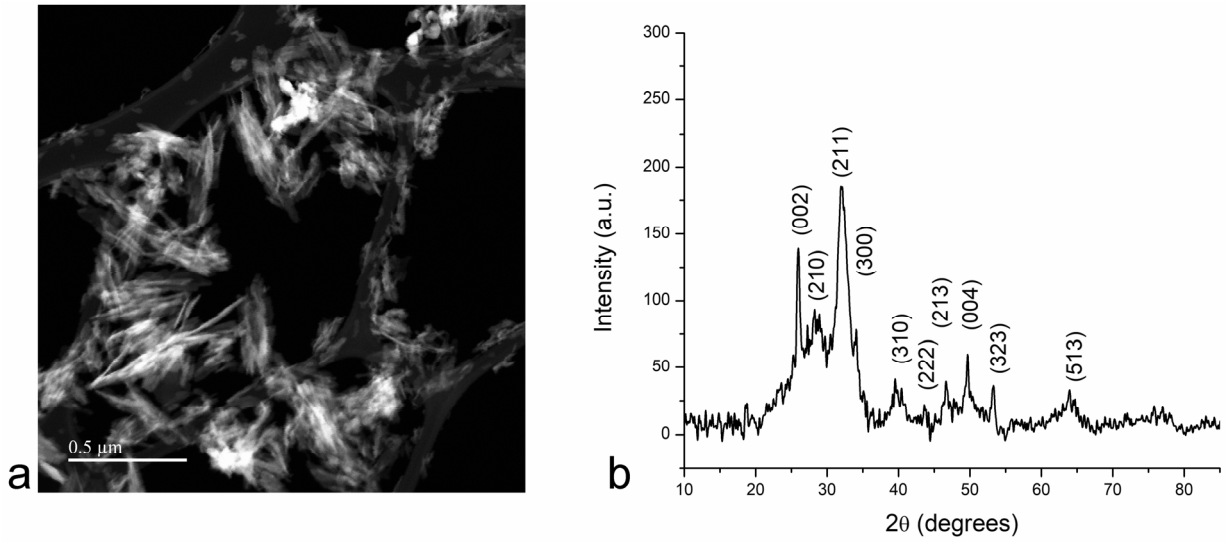

Figure 1. STEM image (**a**) and X-ray diffractogram (**b**) of CaP nanoparticles used as a non-viral transfection agent. Reflections marked with their corresponding Miller indices in (**b**) originate from the hydroxyapatite crystal structure (JCPDS No. 09-0432).

The effect of the same two methods, one of which involved the suppression of p53 expression in primary fibroblasts and the other one of which involved the augmentation of the expression of RUNX2 in cancer cells, on the tumor growth is shown in Figure 3 for the direct co-culture model, where the two types of cells grew homogeneously mixed with one another in a 3D spheroid. According to the results presented in Figure 3, the growth of the cancerous tumors comprising p53-inhibited fibroblasts, as measured by the tumor migration rate, was suppressed on all three days of the experiment, from day 1 to day 7, as compared to the untransformed control co-culture (Day 1: p53 p = 0.0533 vs. control; Day 4: p53 p = 0.3741 vs. control; Day 7: p53 p = 0.0610 vs. control). As for the tumors comprising RUNX2-transfected cancer cells and unmodified fibroblasts, their growth was suppressed relative to the untransformed control co-culture on days 4 and 7 only (Day 1: RUNX2 p = 0.1872 vs. control; Day 4: RUNX2 p = 0.4817 vs. control; Day 7: RUNX2 p = 0.1860 vs. control). Clearly, under the conditions enabling the direct physical contact between the primary cells and the cancer cells, p53 suppression of the normal cells appears to have a more favorable effect than the augmentation of RUNX2 in cancer cells, which is different from the trend observed in indirect co-culture. A previous study on silencing the mRNA expression of p53 showed the reduced migration rate to be one of the consequences of this silencing [58]. Hence, it is possible that by downregulating p53 in primary cells, they become less migratory and start to more tightly interface with the tumor, preventing its excessive growth.

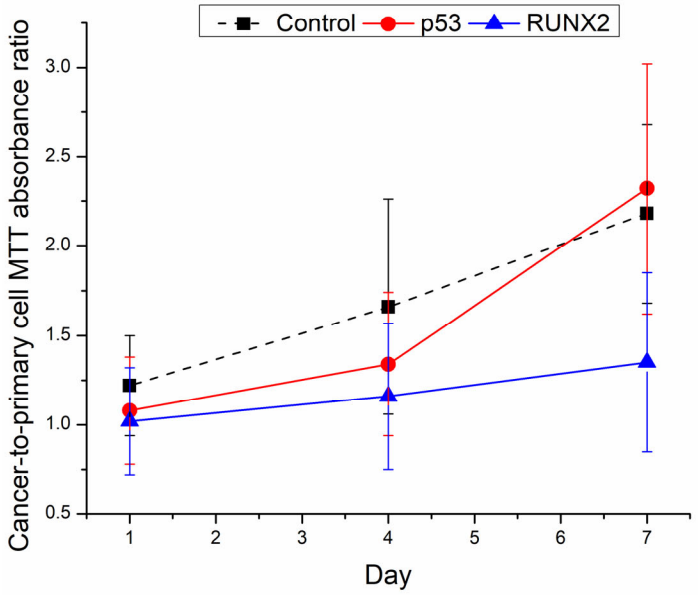

Figure 2. Cancer-to-primary cell MTT absorbance ratio after 1, 4 and 7 days of incubation in the transwell model of the co-culture, where either the primary cells underwent the p53 silencing treatment or the cancer cells were transfected with RUNX2, as compared to the control co-culture, where neither the cancer cells nor the primary cells were treated. Data points represent averages, while error bars represent standard deviation.

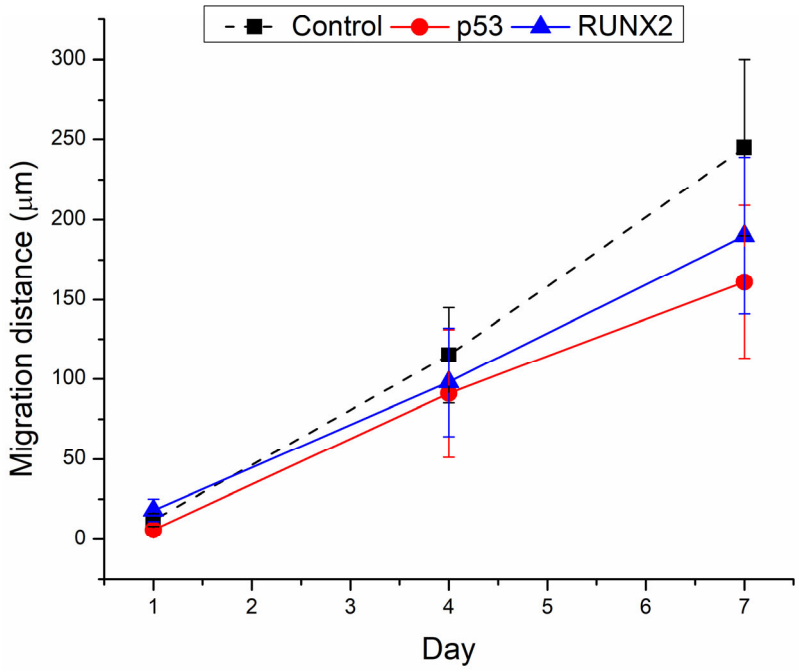

Figure 3. Time-dependent migration distance of spheroids containing primary cells that underwent the p53 silencing treatment and of spheroids containing cancer cells transfected with RUNX2 as compared to the control co-culture, where neither the cancer cells nor the primary cells were treated. Data points represent averages, while error bars represent standard deviation.

However, concerning the precise mechanistic aspects of the curious and rather counter-intuitive effect witnessed here, it is difficult to pinpoint with certainty what may be causing it at the molecular scale of intersecting genetic, epigenetic, metabolic and signal transduction pathways. What is certain, however, is that the aforementioned interfacial nature of cancer is at play here. Given its fundamentally recursive, feedback nature, which has been

confirmed with respect to numerous molecular and cellular crosstalk processes [59–61], it is naturally anticipatable that empowering one side of this interface would empower the other. This is to say that trying to eradicate the tumor residing on one side of this interface may naturally obliterate the healthy cells residing on the opposite side of it as well and, with them, potentially the whole organism. In contrast, empowering one side of the interface may end up empowering the other. Supporting this argument, there have been numerous studies demonstrating enhanced tumor growth when the immune response is overly intense [62–64] and, in turn, tumor inhibition when certain components of the immune system in the tumor microenvironment are suppressed [65,66]. The tumor lysis syndrome, caused by the rapid destruction of tumors during chemotherapy and leading to poor patient response and relapses after the treatment [67], is yet another effect suggestive of the need for a gentler and more pacific approach to mitigating cancer growth than that of its uncontrollable destruction. Unfortunately, in the absence of a more elaborate mechanistic model, the discussion here remains limited to these few systemic remarks stemming principally from the autopoietic nature of biological systems *per se* [68].

To the rather modest amount of evidence provided here, it can be added that this has primarily been a conceptual, mental gymnastics study, where a new direction of research in oncology is being proposed and a new concept is shown to be preliminarily feasible. From here, there are innumerable ways in which the broad avenue for research proposed here could branch out. One obvious way would be to expand the repertoire of cell lines beyond those of E297 glioblastoma cells and primary kidney fibroblasts, as well as to diversify the apoptotic pathway regulators and enhancers of tumorigenesis beyond those of p53 and RUNX2, respectively. In such a way, the procedural scope of the study would be broadened, and the conditions would be provided for the derivation of a better insight into the prospects of the radical idea proposed here. Furthermore, instead of the mitigation of the expression of a protein acting as a tumor suppressor in a healthy cell, there are a plethora of "mean" genes that could be imparted onto it or other "good" ones that could be silenced. Such an addition of undesirable genes to healthy cells of the tumor microenvironment was not explored in this proof-of-concept study, but this presents only one of many prospective options conforming to the general idea elaborated here. In addition, the traits suggestive of loving cancer could be added to or erased from both cancer cells and primary cells by using gene editing tools, such as those enabled by the CRISPR/Cas9 technology. Triggering the autodestruct mechanism in a cell by these means is tempting, but one challenge to keep in mind here is the timing of self-destruction because CRISPR/Cas9 requires generations of cells across many passages to be sorted out, which necessitates sensitivity to a particular marker triggering the autodestruct mechanism. In theory, such edited cancer or healthy cells would be injectable into the tumor area or, in the case of cancer cells, even allowed to spontaneously metastasize in certain areas, assuming that this would have a therapeutic effect analogous to vaccination and not an adverse one, either locally or systemically. Achieving the extension of telomeres, which shorten with cell division, thus decreasing the life expectancy with age, is also not unthinkable at this stage and with these tools in hands.

In general, the rather provocative and counterintuitive approach to cancer therapy proposed here could be augmented and enriched with different molecular effects and empirical methods than those examined here. It could also be tested more thoroughly than in the present report, the data for which were accrued over half a decade ago, but because of the impossibility of garnering funding for this study, it never advanced past this preliminary stage. Lest the idea proposed here grow old and in order for it to be shared in a relatively timely manner with the medical research community, it is presented here in a rudimentary experimental form, for which fellow researchers with more abundant resources at this point must forgive the author. The motivation for the release of this model and these results in this fragmentary form comes from the wish to inspire fellow scientists who do have resources for continuing to explore this cornerstone for a new paradigm and who do harbor enough humanness and sensibilities inside them not to be turned down by its strong analogy with a nonscientific phenomenon called love to continue exploring it. As

for this author, chances are slimmer and slimmer that there will ever be a chance to continue conducting research along the idea of treating cancer through love for it condensed down to the levels of cell and molecular biology, and so the torch is passed on this occasion.

5. Conclusions

With the advent of cancer immunotherapies, it has been implicitly recognized that cancer, mechanistically, is not only an intracellular mutation issue, but that it is, even more so, an interfacial issue, for which the understanding of the interaction between cancer cells and normal cells of the tumor microenvironment and beyond is imperative. The way in which pharmacologic interference with this interface with the objective of treating cancer is approached can be inspired by analogies with scientific or nonscientific phenomena alike. Here, one such provocative nonscientific analogy is being proposed as an avenue worth exploring in the attempt to come up with new forms of oncological therapies, which may overcome the seemingly impassable impasses encountered in the treatment of many forms of cancer today. As per this analogy, the treatment of cancer begins from embracing cancerous tissue as an object of affection instead of a target for immediate eradication, assuming that, in such a way, a harmonious interface between the cancer and its microenvironment will be created, which would help the malign tissue lose its aggressively proliferative properties and halt its growth.

As a rudimentary proof of this new concept, brain cancer cells were co-cultured with primary fibroblasts, and it was shown that both the suppression of a pro-apoptotic factor, p53, in healthy cells and the augmentation of RUNX2, a promoter of tumorigenesis, in cancer cells reduced the growth rate of the neoplasms relative to the growth of the healthy cells in their environment. Here, the suppression of p53 in healthy cells was performed as one analogy of love, which is that a subject in love takes upon itself the traits of the object of one's affection—in this case some of the resistance to apoptosis possessed by the cancer cell. The augmentation of RUNX2 in cancer cells was performed as another analogy of love, which is to give the object of one's affection what it craves and what it needs to grow—in this case the ability of the cancer tissue to proliferate at an even faster rate than it normally does.

Most importantly, these two experimental models are but crude and simplistic methods derived from the notion that cancer is to be loved. However, with the continued exploration of this fundamental idea, more effective treatment strategies may eventually emerge as compared to those rooted in violent, militaristic analogies that dominate today's approach to cancer management. Although the concept presented here will certainly be ridiculed by the purists and the positivists, there is a hope that it may become the foundations for a new paradigm in the field of anticancer therapy if it gets to be further developed and rigorously tested under various biological conditions.

To conclude, this is only the beginning. It is throwing a pebble onto a road that is not there yet. The vision of it, though, is here, and that is the beginning of every dream come true. The direction in which this road may take medicine and humanity is unforeseeable, but it should be explored with ardor and faith. Let this be the final message of this paper, where a radical new concept for cancer therapy is being presented.

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