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Review

Could the biological robustness of low level laser therapy (Photobiomodulation) impact its use in the management of mucositis in head and neck cancer patients

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SUMMARY

Low level laser therapy (LLLT) has been noted to be effective in mitigating the development of oral mucositis among patients being treated with chemoradiation for cancers of the head and neck. To explain the biological basis for this observation we performed a comprehensive literature search. Our investigation identified a substantial number of LLLT-activated pathways that have been strongly associated with negative tumor outcomes including proliferation, invasion, angiogenesis, metastases and cancer-treatment resistance. In light of these findings, we suggest an investigational strategy to assure that LLLT's anti-mucositis efficacy is independent of its possible potential to enhance threatening tumor behaviors. Included are appropriate pre-clinical modeling, short- and long-term follow-up of LLLT-treated patients, and the requirement for consistency of LLLT parameters.

Introduction

Low level laser therapy (LLLT) was first introduced for potential clinical applications by Mester in the late 1960s. This form of laser treatment, currently referred to as photobiomodulation (PBM), limits radiation intensity by transferring low energy to tissues and thereby does not generate heat. Among the clinical indications for which LLLT has been reported to be efficacious are pain relief (back and neck, orthodontic, shoulder), wound healing, carpal tunnel syndrome, colorectal cancer, elbow disorders, fibromyalgia, lymphedema, musculoskeletal dysfunction, myofascial pain syndrome, neurological dysfunctions, patella-femoral pain syndrome, rheumatoid arthritis, shoulder impingement syndrome, and tinnitus. It has also been reported to be useful in the treatment of a number of oral or perioral diseases including dentin sensitivity, alveolar osteitis, osteonecrosis, dental extraction wound healing, aphthous stomatitis, lichen planus, herpes labialis, xerostomia, trismus and mucous membrane pemphigoid. Among its other clinical applications, the potential of LLLT to effectively mitigate oral mucositis associated with anti-cancer drug or radiation regimens has achieved significant attention. There exists a substantial body of data in which the course, severity or incidence of mucositis has been favorably impacted by LLLT [1]. In fact, the possible use of LLLT as a mucositis intervention is noted in the most recent Multinational Association for Supportive care in Cancer/International Society for Oral Oncology (MASCC/ISOO) guidelines [2].

As with any treatment for a cancer supportive care indication, it is critical that the intervention used to mitigate cancer-treatment associated normal tissue injury does so at no cost to tumor treatment efficacy or, worse, does not enhance the malignant potential, local growth, or invasion of the primary tumor. Although investigations evaluating these questions with respect to LLLT are limited and have had contradictory results [3–6], it has been largely assumed that LLLT posed no threat to either tumor behavior or responsiveness to treatment. However, as data accumulates on LLLT's biological pleotropism, this supposition needs to be critically

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evaluated, especially in the case of the application of LLLT to tissue within or contiguous with a tumor field [i.e. head and neck cancer (HNC) and oral mucositis; breast cancer and dermatitis], as it is now apparent that many of the biological activities initiated by LLLT have been associated with enhanced tumor growth, metastases or resistance to treatment.

As with any intervention used to prevent or treat cancer regimen-induced normal tissue injury, there are four critical questions to be answered:

1. Could PBM using LLLT impact tumor growth or proliferation?
2. Could PBM using LLLT effect the risk of local invasion or metastases?
3. Could PBM using LLLT negatively affect a tumor's treatment response (particularly radio-resistance in the case of radiotherapy (RT) in HNC)?
4. Is it possible that the local application of LLLT could have effects distant from the targeted site?

LLLT is biologically robust

Given reports of its clinical diversity, interest in LLLT's ability to impact biological processes has spawned a myriad of studies [7], which provide compelling evidence as to the biological diversity with which LLLT might influence clinical outcomes. LLLT's biological activities have been described in a diverse range of cell and tissue culture systems, animal models, and occasionally in humans. A wide range of cells and tissues have been studied, some normal, some of tumor-origin. However, there has been no uniformity in the instruments tested, characteristics of the laser used (or energy density}, or duration and frequency of exposure [8].

Methods

To better understand the potential relationship between LLLT's biological portfolio and how it might interact with tumors and their treatment we performed a three-phase literature review. Using a broad and undirected initial search strategy for which "low level laser therapy" was the single keyword, those papers describing any biological activity associated with LLLT were noted. A second undirected search was performed using the terms "cancer," "oral cancer," "head and neck cancer," and "pathways" to garner a general list of pathways of potential interest. The search was then honed by querying LLLT with specific pathways noted to be relevant to adverse cancer behaviors including proliferation, infiltration, metastases and resistance to treatment. The final list of references was thereby generated by repeated PubMed queries in which the selection of keywords was directed by the sequence of discovery of terms from one paper to the next.

The result of this search yielded 54 manuscripts describing LLLT-associated biological activities, and 50 manuscripts describing cancer-associated pathways deemed to be relevant to LLLT.

Activities attributed to LLLT

Proliferation

The observation that LLLT may have clinical utility in accelerating wound healing has been largely attributed to its ability to stimulate cell proliferation, cell motility and angiogenesis, while tempering the inflammatory response. The molecular mechanisms by which these occur are still under investigation, but there is now a large body of data from which there seem to be consistent findings relative to LLLT activity.

LLL application increases cellular ATP levels, probably by conversion of absorbed light energy to metabolic energy [9]. Endogenous cell chromophore photosensitization results in reactive oxygen species (ROS) accumulation, which is associated with activation of many pathways [10]. Zhang et al. proposed a scheme wherein ROS generated by LLLT-exposed Hela cells (3–50 J/cm^2, power density 64.6 mW/cm^2) activate Src kinases. This provides a mechanism for LLLT activation of downstream kinases, including MAPK, Akt, PKC and EGF receptor. Interestingly, Src activation is associated with NFκB activity and changes causing proliferation, survival, migration and attachment [10].

One of the most studied pathways impacted by LLLT is PI3K/Akt/mTOR activation, which is associated with cell growth, proliferation, differentiation and survival. LLLT-induced PI3K/Akt/mTOR pathway activation has been reported in normal, dysplastic and cancer cells, and mesenchymal stem cells (MSCs). Furthermore, LLLT dose-dependent activation of Akt (serine/threonine protein kinase), a key regulator of cell growth, proliferation and survival [11] is PI3K-dependent since PI3K inhibition successfully blocked Akt triggering [12].

LLLT also impacts the PI3K pathway at the genetic level. Stem cells derived from bone marrow, dental pulp, periodontal ligament and adipose tissue show LLL-induced proliferation [13–15]. To elucidate biology of LLL-induced mesenchymal stem cell proliferation, Wu and colleagues studied changes in MSC gene expression isolated from rat marrow, using 0.5 J/cm^2 and microarray analysis. Gene ontology identified 119 differentially expressed genes impact by LLLT involving cell proliferation, apoptosis, the cell cycle and other cellular functions with good correlation between Wu's study [16] and one reported by Zhang on LLL-exposed fibroblasts. In both studies, about 18% of differentially expressed genes were associated with proliferation, apoptosis or transcription. Importantly, the authors report 5 PI3K/Akt/mTOR pathway-associated genes (Akt1, Ccndl, Pik3ca Ptpn6, Stk17b) to be potentially important players in LLLT-mediated MSC proliferation. Given the impact of MSCs on tumor behavior, this observation may be relevant in tumor LLLT exposure.

In reviewing LLLT-associated mechanisms driving cell proliferation, Goa and Xing note that LLLT phosphorylates hepatocyte growth factor (c-Met, a tyrosine protein kinase receptor) during proliferation, and that TPKR may activate a number of pathways associated with proliferation—including Ras/Raf/MEK/ERK and PI3K/Akt/elF4e and PI3K/Akt/eNOS. They also associate proliferative activity with ROS/Src (see above) and $\Delta\Psi$m/ATP/cAMP/JNK/AP-1 [17]. Studying human-derived glioblastoma cells, Fukuzaki et al. noted that combining a γ-secretase inhibitor with LLL increases cell proliferation via Akt activation [18].

While studies mostly report LLL-induced cell proliferation, findings are not uniform, perhaps influenced by the variations in cell lines tested, culture conditions and timing of LLL exposure, and differences in LLL fluence, energy, and density. For example, Schartinger reported [19] that LLL stimulated fibroblast proliferation, but decreased similar changes in human bronchial epithelial cells and in a human oral squamous cell cancer (SCC) cell line. In contrast, Sperandio et al. [20] reported increased proliferation and expression of cyclin D1 in LLL-exposed (3 J/cm^2 to 12 J/cm^2) human keratinocytes (HaCaT cells). In another study, the same authors studied the effects of LLL (2.05, 3.07 or 6.15 J/cm^2) on oral dysplastic cells or oral SCC cells. Similar to previous reports, they noted Akt/mTOR/Cyclin D1-mediated modulation of cell growth, expression modification of proteins associated with progression and invasion of the cell lines, and pAkt, pS6 and cyclin D1 with production of an aggressive Hsp90 isoform [21]. Likewise, Gomes Henriques et al. [22] reported LLL-exposed (0.5 J/cm^2 or 1.0 J/cm^2) cultured human tongue SCC cell lines increased proliferation in both laser energy groups after 24 h of culture with the higher dose being significant. This increase coincided with increased cyclin D1 and nuclear β-catenin expression, while reducing E-cadherin and induction of MMP9.

Differences in fluence could be a cause for the inconsistencies in reported LLL-associated biological signals. Studies reported by Gao compared effects of low and high energy LLL. Using protein kinase C's as a surrogate endpoint given their involvement in cell proliferation, tumor promotion, differentiation and apoptosis, the response of human lung adenocarcinoma cells to energy levels of 0.8 J/cm^2 or 60 J/cm^2 were compared. Whereas lower energy LLLT-induced cell proliferation by specifically activating PKCs, they found that PKC activity decreased during apoptosis induced by high fluence laser [17]. Similarly Al-Watban and Andres [23] found that the optimum biostimulatory dose of LLL was 180 mJ/cm^2 compared to bioinhibitory activities at doses of 420–600 mJ/cm^2.

Further differences in response to low or high fluence were noted by Pellicioli et al. In the study of oral epithelial cells, they found that in contrast to 20 J/cm^2, 4 J/cm^2 resulted in ROS accumulation that was not damaging to DNA and did not induce genomic instability. Interestingly, when comparing the same two energy levels in oral keratinocytes, both energy levels contributed to epithelial cell migration through activation of the mTOR signaling pathway [24].

Connective tissue TGF-β/Smad signaling

LLLT's favorable effects on collagen synthesis manifests clinically in wound healing and cosmetic applications. Dang et al. [25,26] note that energy differences impact this effect's mechanisms. While higher energy (60 J/cm^2) implicates the TGF-β/Smad pathway, lower energy lasers influence interleukin-6 (IL-6) and heat shock protein 70 (Hsp70) activation.

Angiogenesis and cytokines

LLLT's healing properties may be related to its influence on angiogenesis and biologically active cytokines. Reports support LLLT's ability to induce angiogenesis (through numerous pathways) and enhance the production of various biologically potent mediators (Table 1).

By treating the bronchus skin of an *in vivo* rat model with LLL (5.4 J/cm^2), de Lima and colleagues observed reduced ROS formation, increased GSH, HSP70, and PPARγ levels [27]. Using a lower energy (0.2 J/cm^2) to treat burns in rats, Gupta et al. noted that LLLT decreased TNF-α and NF-κB levels, but upregulated protein levels of VEGF, FGFR1, HSP60, HSP90, HIF-1α and MMP2 and MMP9, while stimulating cellular proliferation [28]. A summary by Kim and Calderhead [32] noted 24 growth factors, interleukins, inflammatory cytokines, and small molecules that are reportedly activated by LLLT include 9 growth factors (BNF, GDNF, FGF, bFGF, IGF-1, KGF, PDGF, TGF-β, and VEGF), 5 interleukins (1α, 2, 4, 6 and 8), 4 inflammatory cytokines (PGE2, COX2, IL-1β, and TNF-α [note that TNF activation conflicts with Gupta's reports]) and 6 small molecules (ATP, cGMP, ROS, Ca^{++}, NO and H^+). The biological activities attributed to aforementioned molecules range from proliferation and migration, to angiogenesis and pain relief.

Given the importance of angiogenesis in wound healing, Feng and her colleagues investigated LLLT-mediated upregulation of VEGF expression. LLL-exposed (doses of 0.3–2.1 J/cm^2) human umbilical vein endothelial cells exhibited enhanced ERK-dependent Sp1 activity, ERK activation and nuclear translocation from cytoplasm, and, not only increased VEGF expression, but also G1-to-S phase transition and proliferation through activating ERK/Sp1 [29]. Of note, MAPK activation as part of LLLT-production of VEGF was demonstrated in an immortalized granulosa cell line [30] using GaAlAs irradiation (60 Mw, 830 nm, 60 s). Increased MAPK activity increased VEGF expression.

To uncover the mechanism by which LLLT impacted cell proliferation and wound healing, Safavi et al. studied the impact of He–Ne laser treatment (7.5 J/cm^2) to gingival and mucosal tissue in rats by evaluating selective cytokine gene expression [31]. Whereas expression of IFN-γ and IL-1β was significantly blunted, the expression of PDGF and TGF-β was dramatically increased, but neither TNF-α or bFGF expression was impacted.

The lack of modification in bFGF expression contrasts with the findings of Kim and Calderhead [32], Sharifian et al. [33] who used 0.2 J/cm^2, Esmaeelinejad and Bayat [34] who studied human fibroblasts exposed to three doses of 0.5 J/cm^2, 1 and 2 J/cm^2, or Usumez et al. [35] who evaluated the effects of LLLT in a rat mucositis model. These authors also reported increases in PDGF. Likewise, by exposing human vascular endothelial cells to LLL (2–8 J/cm^2), Szymanska et al. [36] reported angiogenesis at the expense of decreased VEGF concentration, suggesting active VEGF involvement. TGF-β secretion was also dependent on the source wavelength used.

Lastly, LLLT impacts the signaling cascade of NF-κB, an important driver of the inflammatory mediators. Although its exact impact is inconclusive, several studies suggest that LLLT downregulates NF-κB transcriptional activity [16]. NF-κB signaling may be initiated by ligation of toll-like receptors, leading to recruitment of the TIR domain proteins MyD88, TRIF and TIRAP [16]. By irradiating adipose stem cells with LLL (8 J/cm^2), Wu et al. found that LPS-induced pro-inflammatory cytokine (IL-6, 1β, 8 and Cox-2) production was inhibited, probably through increases in intracellular cAMP levels. Similarly, Song et al. reported LLLT-induced (20 J/cm^2) activation of Src/Syc kinases, which phosphorylate MyD88 and attenuate the TLR-mediated inflammatory response

Table 1
Biological mediators associated with LLL exposure. The biological activities associated with LLL exposure are diverse and robust. Many are associated with LLL stimulation of molecular intermediaries which fall into a variety of classes as noted. The actions and effects associated with the mediator classes are not intended to be comprehensive. Rather, they provide insight into those activities that seem most relevant to LLL and tumor behavior and/or response to therapy. Elements of this table were based on Kim and Calderhead [32].

Mediator classification	Molecules	Action and/or effects
Growth factors	BNF, GDNF, FGF, bFGF, IGF-1 KGF, PDGF, TGF-β, VEGF	Proliferation Differentiation Angiogenesis Migration Chemotaxis
Anti-inflammatory cytokines	IL-2, IL-4, IL-8, IL-10	Differentiation Proliferation Immune activation Chemotaxis Angiogenesis
Pro-inflammatory cytokines	IL-1α, IL-1β, IL-6, TNF-α, PGE2, COX2	Stimulate and accelerate inflammation Proliferation Angiogenesis Promote migration Anti- and pro-apoptosis
Heat shock proteins	HSP70, HSP90	Chaperone protein Enhanced cell survival Tumor growth
Matrix metalloproteinases	MMP2, MMP9	Cell survival Prevent terminal differentiation Tissue remodeling
Small molecules	ATP, GSH, ROS, Ca^{++}, NO, H^+	Normalization of cell function Migration Angiogenesis Proliferation

in microglia [37]. This reduced cytokine expression and NO production in LLL-exposed cells. As mentioned previously, Zhang et al. propose ROS accumulation as a mechanism of LLLT-induced Src kinase activation [10].

Effects on apoptosis

Contrasting its reported effects on cell proliferation, LLLT has been noted to impact apoptosis through a number of different mechanisms.

Staurosporine, a naturally occurring antibiotic capable of inducing apoptosis, was inhibited by LLLT through inactivation of the GSK-3β/Bax pathway. In studying LLLT inhibition of staurosporine-induced apoptosis, Zhang et al. [38] irradiated human lung adenocarcinoma cells (1.2 J/cm^2). As previously noted, the same authors reported that LLLT-induced activation of PI3K/Akt pathway promotes proliferation. In this case, they found that LLLT suppressed Bax translocation and caspase-3 activation through the PI3K/Akt/GSK-3β pathway.

In a different model where promotion of skeletal muscle cell activation and proliferation was observed, Shefer [39] found that LLLT enhanced the expression of Bcl-2 and impeded the expression of BAX. The finding supported an anti-apoptotic effect given the robust increase in Bcl-2 and the decrease in BAX. Furthermore, the authors noted a reduction in p53 and cyclin-dependent kinase inhibitor p21.

Amyloid-β-peptide ($A\beta_{25-35}$) is known to cause cell apoptosis both *in vitro* and *in vivo* [40]. Yes-associated protein's (YAP) pro-apoptotic activity is mediated by p73. In a recent study using cultured pheochromocytoma cells (PC-12), Zhang and Wing noted that LLLT at a dose of 2 J/cm^2 activated the Akt/YAP/p73 pathway by promoting YAP cytoplasmic translocation and thereby inhibited apoptosis. Furthermore, they noted that this effect could be blocked by inhibition of Akt expression.

Finally, Chu et al. [41] found that high fluence LLL radiation (120 J/cm^2, a dose that is higher than typically used clinically) upregulated survivin, an inhibitor of apoptosis.

LLLT and micro-RNA's

Noted to affect tumor behavior and treatment response, miRNA's are also modified by LLL irradiation [42]. Wang et al. noting that LLLT impacted mesenchymal stem cell (MSC) proliferation, hypothesized involvement of miRNAs in LLLT-stimulated MSC proliferation. Therefore, they used cultured rat and human MSCs to profile miRNA response to 0.5 J/cm^2 of laser energy. While they reported LLLT-induced upregulation of 19 miRNAs and down-regulation of another 15, 6 of the more broadly conserved miRNAs identified were associated with 102 possible prediction-related target genes. Of these, miR-193 was the most upregulated and, probably by regulating CDK2 activity, was an important driver of MSC proliferation [43].

Distant (abscopal-like) effects

Whether there are systemic consequences to focal LLLT therapy is unresolved and studies demonstrated conflicting results. In a first study, Fronza exposed the mandibular extraction sockets to 13 days exposure of energies of 6 J/cm^2 and measured serum triiodothyronine and thyroxine levels as surrogates of thyroid function [44]. They were unable to detect any difference in pre- vs. post-irradiation levels. In contrast, also using rabbits, the authors assessed daily doses of 5, 10 or 20 J/cm^2, administered every other day for 7 sessions. In contrast to their first study, the authors reported statistically significant increases in T3 and calcium in all groups, and concluded that LLLT applied to the mandible impacted thyroid function [45].

Using a more circumspect approach, Hentschke et al. exposed the gastrocnemius to 3 or 21 J/cm^2 for 10 days in a rat heart failure model [46]. They then evaluated peripheral blood levels of an inflammatory cytokine panel and detected changes in IL-6, TNF-α and IL-10. They concluded that LLLT was able to induce, not only focal changes in radiated muscle, but also systemic effects.

Could LLLT contribute to tumor growth and proliferation?

Many of the pathways discussed herein have been strongly implicated in adverse cancer behaviors (Fig. 1). The PI3-Kinase pathway was first associated with cancer almost 30 years ago [47]. As a component of the PI3K/Akt/mTOR pathway, it is especially relevant to head and neck cancers (HNC's) where it is frequently mutated [48]. Because of its importance in tumor behavior, the PI3K/mTOR pathway has been identified as a potential target for intervention.

PI3K/mTOR pathway inhibition has proved effective as a radiosensitizer in colon cancer models [49]. Similarly, Leiker et al., using cell culture and animal xenograft head and neck SCC (HNSCC) models [50], found that PI3K/mTOR inhibition preferentially radiosensitized human HNSCC cells compared to normal human fibroblasts. Likewise, the same PI3K/mTOR inhibitor delayed tumor regrowth in a nude mouse xenograft model. Chang also described how the PI3K/Akt/mTOR pathway impacted radiosensitivity (and radioresistance) [51] in radioresistant prostate cancer cell lines. These cells were not only radioresistant, but were also associated with more aggressive tumor invasion and stem cell formation (sphere). Furthermore, the epithelial–mesenchymal transition (EMT) and cancer stem cell (CSC) formation were also correlated with PI3K/Akt/mTOR signaling–blocking which pathway improved radiosensitivity, and reduced EMT and CSC phenotypes.

PI3K/Akt signaling also has the ability to activate downstream pathways and genes, among which is ERK. Significant for cancers of the upper aerodigestive tract, ERK/MAPK-activation of EGFR, a component of LLLT activity (Kawano 2012), has been identified as having potential importance in tumor progression [52], probably through the promotion of VEGF and endothelial cell proliferation [29].

Although a direct relationship between LLLT and epithelial adhesion molecule (EpCAM) has yet to be explored, EpCAM's association with the PI3K/Akt/mTOR pathway has been well-documented [53] and is relevant to the current discussion. EpCAM is a transmembrane glycoprotein antigen that was initially described in human colon cancers in the 1990s. It has been described as being common in patients with oral cancers; Yanamoto noted its overexpression in over 60% of tongue cancers, and that its presence was associated with negative tumor outcomes such as size, metastases, invasion and histological features [54]. Similar results were reported using tissue/cells from patients with prostate cancer, as well as EpCAM's ability to attenuate chemo/radiosensitivity [53].

Numerous LLLT-induced anti-apoptotic activities are attributable to LLLT, and these might influence tumor survival and treatment resistance. As previously mentioned, higher energy LLLT implicates the TGF-β/Smad pathway to promote collagen synthesis. This pathway, which normally inhibits proliferation and induces apoptosis, is often inactivated in oncogenesis [55] However, depending on the tumor stage, TGF-β's tumor suppressive roles in early stage tumors changes to promote tumor progression in advanced tumors [56]. In fact, many tumors overproduce TGF-β, which promotes oncological invasiveness by inducing EMT,

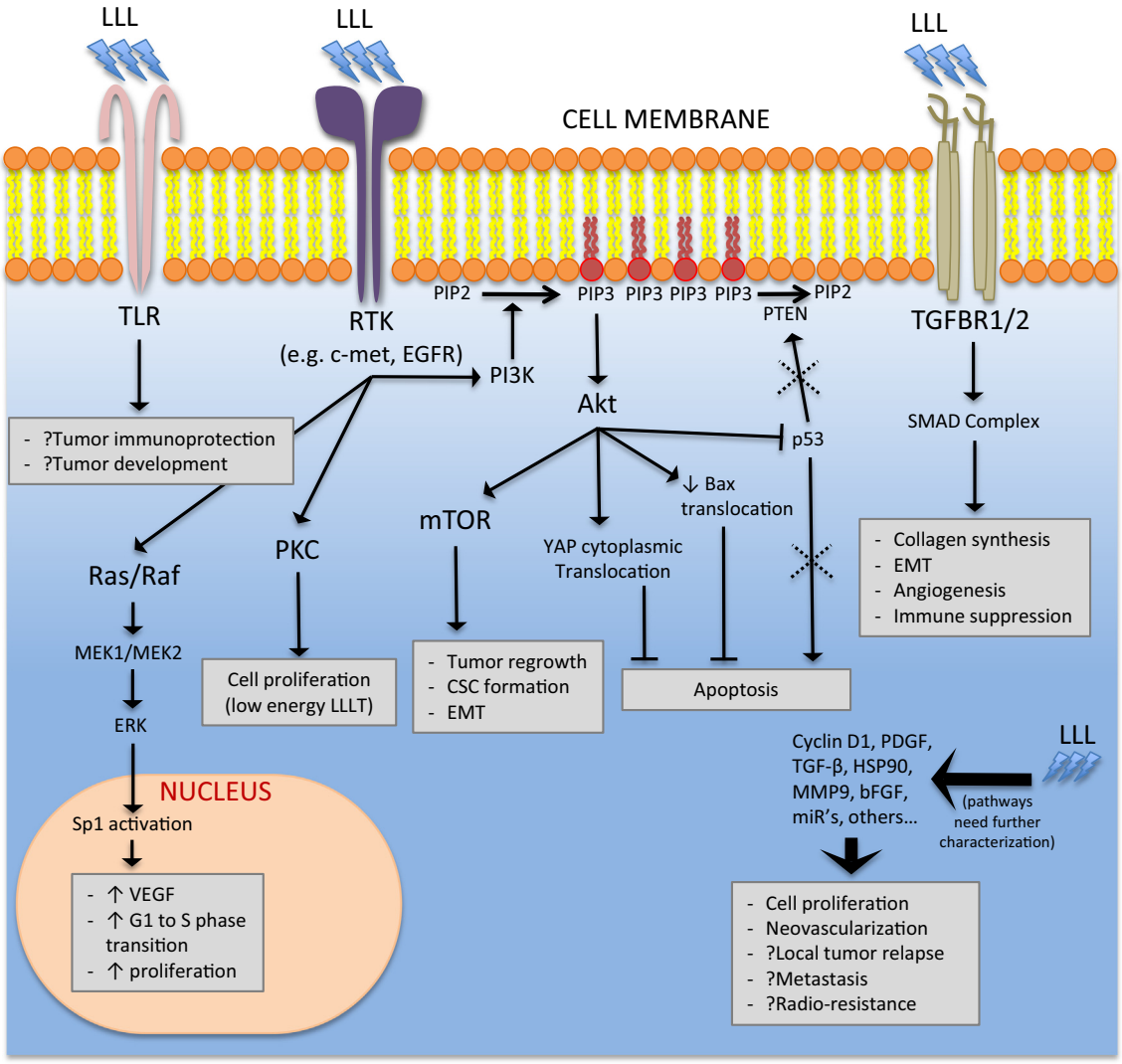

Fig. 1. Overview of some of the pathways associated with LLLT which have the potential to impact tumor behavior or response to treatment. As noted in the text, data predominantly derived from *in vitro* studies show relationships between LLLT and activation of a range of receptors and signaling pathways that have been implicated in undesirable tumor behaviors or response to treatment. This figure provides a selective overview of some of those pathways and their possible consequences. These complement and/or enable many of the biological mediators described in Table 1. Notably, additional *in vitro* and especially *in vivo* studies in which LLLT conditions are standardized will be critical to more definitively define LLLT's biological potential and, critically, the clinical meaningfulness and true risk assessment of its application in head and neck cancer patients.

angiogenesis, and immune suppression [55]. For instance, in their *in vitro* and *in vivo* studies, Hwang et al. observed that TGF-β1 stimulation results in transwell invasion through the Smad signaling pathway, and activation of Src and P38 mitogen activated protein kinase. TGB-β1 was also observed to cause expression of Podoplanin (PDPN), a marker of invasive tumors [56,57]. Furthermore, high Smad6 mRNA expression, which has a blocking effect on TGF-β, is associated with survival in oral squamous carcinoma patients [58].

Could LLLT contribute to the likelihood of metastases, invasion and resistance to treatment?

Many factors which contribute to the migration, invasion and metastatic potential of tumors are stimulated or activated by LLLT.

The relationship between tumor growth and angiogenesis is well established. Vassilakopoulou et al. note that VEGF is highly expressed by head and neck tumors, and is negatively associated with patient prognosis [59]. Many LLLT-inducible cytokines have been implicated in cell proliferation, tumor vascularization, local expansion, and metastases—including VEGF and PDGF [31,35], which are expressed by both HPV positive and HPV negative cell lines [60].

Ninck et al. [61] makes similar reports in cells cultured from 15 anatomically diverse human HNSCC's, including one maxillary sinus neoplasm. By measuring VEGF, PDGF, bFGF, GCSF and GMCSF expression using immunohistochemistry and ELISA, major roles for VEGF and PDGF were suggested. Interestingly, the more cytokines noted, the worse the clinical behavior of the tumor.

Unlike Ninck's study, but consistent with reports of others, Han and colleagues associated tumor bFGF expression with aggressive behavior. Not only was bFGF more highly expressed in human esophageal cancers studied from 79 patients, but the extent of expression was also associated with depth of invasion, lymph-node metastases, and TNM stage [62]). Even in non-neoplastic tissue, bFGF activates the RAS/MEK/MAPK/RSK/BAD pathway to inhibit radiation induced-apoptosis of human umbilical vein endothelial cells [63].

Gupta et al. recently reported that LLLT upregulated HSP-60 and -90 [28] and increased MMPs 2 and 9. Gomes et al. reported similar findings with regards to MMP9. HSP90, a molecular chaperone that

protects a range of protein kinases and transcription factors from degradation [64], is a frequent target for anti-cancer therapy. Its inhibition blunts tumor growth, and improves response to radiotherapy [64,65]. In a study of 87 patients with cancers of the oral cavity, oropharynx, larynx or hypopharynx, Lorenzo et al. noted that expression levels of HSP-90 in tumor specimens correlated with response to radiotherapy. Whereas the local relapse rate was 2.9% in patients with low HSP-90 levels, it was 38.2% in individuals with high expression rates [66]. Consequently, the finding the LLLT upregulates HSP-90 suggests the possibility that laser treatment might promote tumor growth while simultaneously reducing radiosensitivity.

Although strongly implicated with tumor invasion, the exact role of matrix metalloproteinases (MMPs) in oncogenesis is under investigation [67]. While MMP-1,2,3,7,8,10,11,13,14 and others have roles in the invasion of HNC's, MMP2 and 9 are most often discussed and targeted [67–69] reported MMP9 to be critical in tumor neovascularization and metastases of oral SCC's. Similarly, Jia noted that miR-34a impacted nodal metastases of tongue cancer by targeting MMP-9. A retrospective review of 145 HNC patients by Gunawardena et al. [70] revealed that tumor-expressed MMP2 negatively prognosticated survival. In a meta-analysis of 33 studies that included 10,516 cases, Liu et al. [71] reported that MMP-1,3,7 and 9 may be associated with cancer metastases, but conclusions on MM2 required additional study. Altogether, LLLT's exacerbation of MMP activation might support tumor invasiveness and/or mitigate cancer therapy response.

Survivin, a protein member of the inhibitor-of-apoptosis family is upregulated by high fluence LLL radiation [41]. The molecule promotes cell proliferation, inhibits apoptosis, increases PI3K/Akt- and ERK signaling, and decreases PTEN function. Studying human lung adenocarcinoma cells, Chu et al. noted that LLL radiation induced survivin, and impacted tumor survival following survivin activation. Likewise, Ettl and colleagues recently described differences between radiosensitive and radioresistant HNC cell lines, and noted that the radioresistant cell line was characterized by survivin upregulation [72].

Lastly, while MyD88 expression is associated with decreased tumorigenesis in certain tumors, such as prostate cancer [73], other groups like Koch et al. [74] report that MyD88 signaling may contribute to treatment-resistance in HNSCC. Further studies of MyD88 tumorigenesis pathways and its role in various cancers is needed. Lastly, Szczepanski et al. noted that triggering TLR 4 expressed on HNSCC promoted tumor development and was immunoprotective of the tumor [75].

Could LLLT have a systemic or distant, abscopal-like effect?

No conclusive evidence suggests that LLLT may impact tissue distant from the site of application. Weber [45] reported contradictory results when he applied LLLT of different energies in a rabbit model, and assessed changes in thyroid function [4,45]. In support of LLLT abscopal activity, Hofling et al. [76] detected significant increases in serum levels of TGF-β1 in patients treated for chronic autoimmune thyroiditis with LLLT. Given the reported importance of TGF-β1 in the induction of metastases [77], further assessment of LLLT-induction seems prudent.

Conclusions and commentary

LLLT is a potential intervention for oral mucositis associated with chemoradiation used for the treatment of head and neck cancer and other solid tumours and hematological malignancies. Data derived from clinical trials recognizes this application [78]. LLLT has also been shown to be biologically robust in that its application to a broad range of *in vitro* and *in vivo* test systems reveals a range of activities that exceeds most drugs or biologicals. Conclusions around both LLLT's efficacy in clinical use and biological activities must be tempered by the lack of consistent instrumentation, dosing, energy, and fluence parameters.

The U.S. Food and Drug Administration classifies LLLT devices as Class II laser devices. There is, as yet, no FDA guidance for industry and FDA staff for LLLT applications for mucositis or other regimen-related toxicities. Perhaps because of its classification as a device, or its traditional applications for benign conditions or in healthy patients, the biological implications of focally applied LLLT in patients with cancer have not been studied with the same rigor as is mandated for pharmacological interventions for cancer regimen-related toxicities to assure that with LLLT protection of normal tissues is not occurring at the cost of negatively impacting tumor behavior or mitigating the effectiveness of anti-tumor therapy.

Consequently, the fact that a broad range of biological activities ascribed to LLLT are also associated with negative tumor behaviors and treatment responsiveness requires additional study before LLLT can be advocated in patients with head and neck cancer. Such investigations might include standard pre-clinical *in vitro* and *in vivo* tumor models, short and long term follow-up of LLLT-treated patients as is required for other mechanistically-based toxicity treatments. Retrospective analysis of patients already treated with LLLT might provide some preliminary information, but a well-designed, prospective study is critical. Incumbent on any analysis will be the need for uniformity of the LLLT administered.

It would also be quite reasonable to expect that individual patient's response to LLLT is not uniform. Rather, as is the case with genomic determinants associated with pharmacological forms of therapy, genomics associated with LLLT-affected pathways are likely to modulate patient's responsiveness, both at the level of the tumor and relative to mucosal protection. Like drugs, the criteria for personalized or precision medicine will likely be applicable to LLLT.

A favorable effect of laser therapy in attenuating chemotherapy-induced oral mucositis was first reported about twenty years ago [79,80]. Since then, the results of a number of trials favor its efficacious effect. Simultaneously, other observation of LLLT's clinical impact spurred investigations to answer questions around its mechanism of action and biological prowess. And, acknowledging a lack of uniformity of LLLT systems, doses and conditions used to study its biological activity, there is an abundance of data which suggests that many of the pathways and molecules activated by LLLT have also been implicated in tumor growth, invasion, metastases and resistance to drug or radiation therapy.

Despite these simultaneous paths – clinical trials with LLLTs and mechanistic studies of its biology – there has been no effort to systematically interrogate the relationship between LLLT and tumor behavior and response in standard models or to follow patients treated with LLLT in the context of tumor outcomes (i.e. time-to-progression, survival, RECIST [81] as would be expected of drugs for the same indication or biological profile. Consequently, there is both an opportunity and obligation for investigations in these areas.

Until we have data that definitively establish that LLLT does not adversely impact established cancers, adherence to the North American Association for Photobiomodulation Therapy (NAALT, 82) prescribed contraindication to LLLT use over a tumor site as in the case of HNC seems prudent.

Conflict of interest statement

None declared.

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