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Overview

Is it Time to Change Radiotherapy: The Dawning of Chronoradiotherapy?

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Abstract

A minority of radiotherapy patients experience adverse reactions as a result of the inevitable irradiation of the surrounding healthy tissue. These reactions range in severity and affect the patient's quality of life, as well as being dose-limiting. If the patients most at risk of toxicity could be identified before radiotherapy, the treatment pathway, radiation dose or fractionation could be altered to reduce toxicity while maintaining efficacy. Previous research is described on how chemotherapy treatments could be improved through the delivery of drugs at specific times of the day ('chronomodulation') based on the circadian rhythm. More recently time-of-day effects have been investigated for radiotherapy, yielding complex results, but with some promise for genetic prediction of the optimal time for treatment. This would allow an almost cost-free modification to treatment that would reduce toxicity. Despite the increasing evidence for 'chronotherapy' for treating cancer, little work has looked into the potential mechanisms underlying the time-of-day effect, which potentially include differences in inflammation, cell cycle or hormones. This overview discusses the main findings from chronotherapy so far and comments on why elucidating the biological mechanisms relating radiotherapy toxicity to the circadian cycle warrants further investigation.

Key words: Chronoradiotherapy; Circadian rhythm; Genetics; Toxicity

Statement of Search Strategies Used and Sources of Information

A Pubmed search was carried out using combinations of the following terms: apoptosis, cancer, cell cycle, chemotherapy, chronomodulated, chronoradiotherapy, chronotherapy, chronotype, circadian, cortisol, DNA damage, DNA repair, immunity, melatonin, time of day, toxicity. Additional publications from the reference lists of the original search articles were also looked at.

Introduction

In England, half of all cancer patients receive radiotherapy as part of their treatment (either adjuvant or palliative), contributing to 40% of cancer cures [1]. However, despite technical advances in delivery and planning to target the radiation to the patient's tumour, irradiating healthy tissue is unavoidable. The consequences of damaging healthy cells depend on the tumour location, together with patient and other treatment factors. Adverse reactions range from transient acute effects to organ damage and secondary cancers. Although adverse reactions occur in the minority of patients, acute and late side-effects are dose-limiting and therefore may limit the efficacy of treatment and can affect an individual's quality of life in the years after radiotherapy. The development of side-effects shows variability between patients, influenced by therapeutic factors, environmental factors and genetic factors, with the latter having the potential to predict an individual patient response to radiotherapy.

Over the years, the link between circadian biology and disease has gained traction in the clinic, with increasing interest in studying how the time of day might affect clinical outcomes. Cancer research has found strong associations with an individual's circadian clock and cancer incidence, chemotherapeutic treatment and now radiotherapy

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treatments. Such research has enabled chronomodulated chemotherapy treatments (that is chemotherapy delivered at a particular time of day to coincide with the biological clock) to be delivered in the clinic. Now more research is looking into how the time of day might affect radiotherapy outcomes to see if chronomodulation may be beneficial.

The Circadian Clock

To understand how the time of day might influence cancer therapies, it is important to understand how the circadian clock functions. The circadian clock is an endogenous system found in a wide variety of organisms that allows the body to regulate both behavioural and physiological processes in a rhythmic manner, in response to external cues (Figure 1). In humans, the length (or period) of the clock is approximately 24 h and is reset by light and temperature cycles established from the Earth's rotation [2–4].

Each individual cell and tissue has an endogenous molecular clock, but there is a master regulator in the suprachiasmatic nucleus (SCN) located in the hypothalamus of the brain. It is the SCN that is responsible for synchronising the circadian rhythms of all peripheral tissues in the body using light signals that are picked up by photoreceptors in the eyes. These signals are then transmitted to neurones in the SCN to coordinate a variety of biological functions in peripheral tissues, such as cerebral activity, sleep and wake patterns, cardiovascular activity, renal activity, immune activity, body temperature, blood pressure, metabolism and endocrine activity [2,3].

The importance of the SCN as the central pacemaker of the clock was highlighted when SCN tissue was transplanted into mutant organisms that lacked a circadian rhythm, restoring the clock [2,3]. Further research into the circadian rhythms of peripheral tissues have also shown that the circadian clock can oscillate independently of the SCN for a short while, but quickly become asynchronous without the control of the SCN [2,3].

The cellular molecular clock is made up of a set of core genes that are essential for generating the body's rhythms (Figure 2). The main feedback loop consists of the CLOCK and BMAL1 proteins, whose genes form part of the basic helix-loop-helix-Per/Arnt/Sim (bHLH-PAS) family of transcription factors. The CLOCK and BMAL1 proteins form a heterodimer that can bind to *cis*-regulatory enhancer sequences called E-box sequences. To initiate the transcription of its target genes – Per1, Per2, Cry1 and Cry2 – the CLOCK:BMAL1 heterodimer binds to the E-box sequences located within the promoter regions [2,4]. Upon their

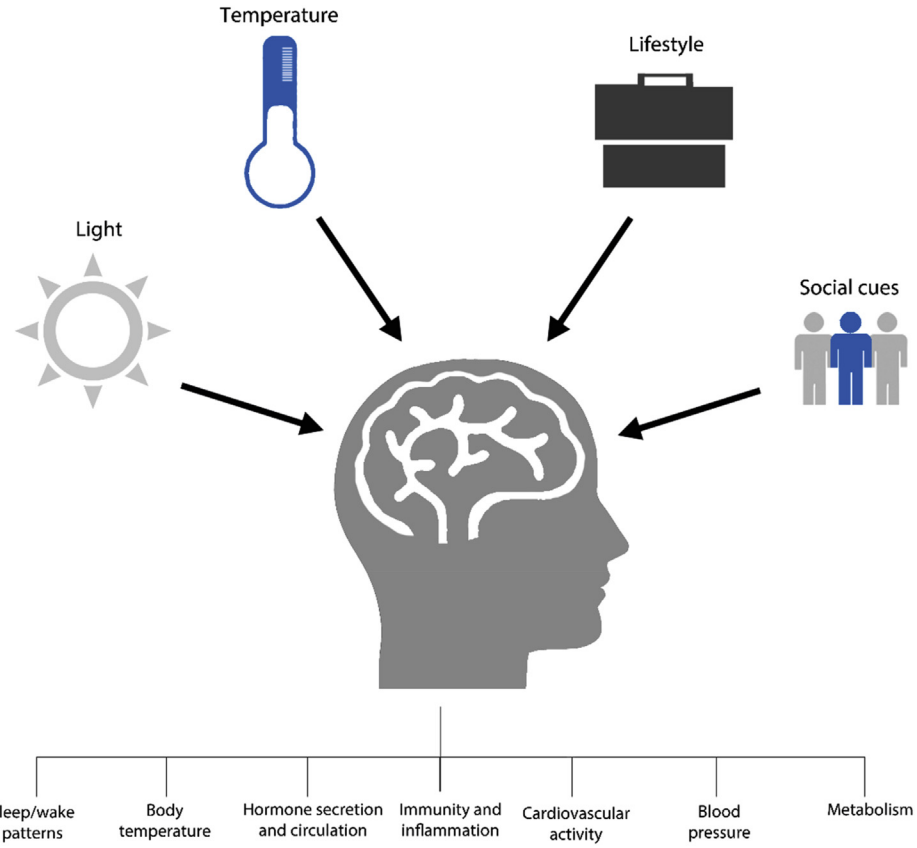

Fig 1. Clock input/output. Factors influencing the clock and the physiological effects this can have on the body. Light is the main input of the circadian cycle, but the clock can also be influenced by temperature and social cues and lifestyle, e.g. shift work. These inputs can all affect signalling in the brain, cardiovascular, metabolic and endocrine systems.

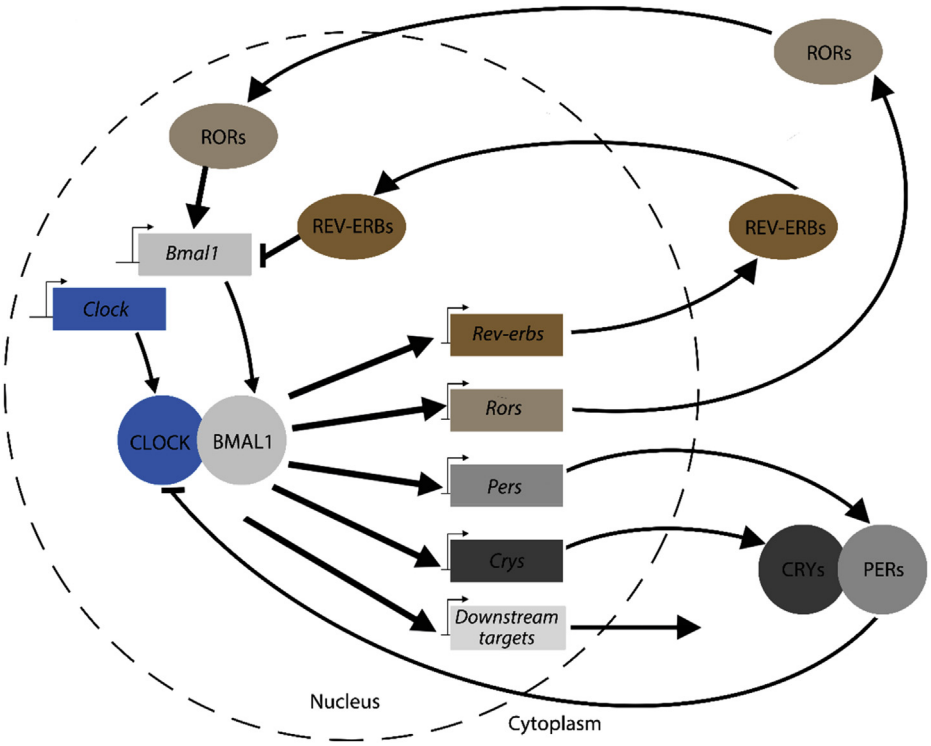

Fig 2. Molecular clock. The core clock components and main transcriptional feedback loops that function in the circadian clock. The CLOCK:-BMAL1 heterodimer initiates the transcription of downstream clock genes to repress its own transcription by the PER:CRY heterodimer and REV-ERBs. CLOCK:BMAL1 is also able to initiate the transcription of non-essential downstream clock genes, such as PER3 and NOC.

translation, the PER proteins form heterodimers with the CRY proteins and are transported back into the nucleus. When sufficient amounts of PER:CRY heterodimers accumulate they can then bind to the CLOCK:BMAL1 heterodimer, repressing their own transcription [2,3]. In addition to initiating the transcription of the Per and Cry genes, the CLOCK:BMAL1 heterodimer can also initiate the transcription of Rev-ERBs (Bmal1 repressor) and RORs (Bmal1 activator) [2–4].

These feedback loops are essential for the regulation of downstream target genes that are not essential for the molecular timekeeping of the clock, but instead function in keeping the body's physiological process in time with the cycle. For example, the circadian clock can lead to the activation of various genes involved in metabolism, such as the RNA deadenylase Nocturnin (NOC) [5–7] and Period 3 (Per3), which has been associated with sleep homeostasis [8–10].

Furthermore, various post-translational modifications can happen to the clock proteins as another method of regulation, such as phosphorylation and acetylation [2,3,11]. There is also a large amount of chromatin remodelling by various histone-modifying proteins during the transcription and translation feedback loops [2].

Chronotype

The variation in an individual's sleep–wake cycle is known as their diurnal preference or chronotype, with individuals being described as showing a tendency to wake early and be more alert in the mornings 'morningness' ('larks') or vice versa 'eveningness' ('owls'). Several researchers have devised questionnaires to be able to assess the nature of a person's chronotype without the requirement for biological samples. The most common self-assessments used today are the morningness–eveningness questionnaire and the Munich chronotype questionnaire [12,13].

Evidence suggests that a person's chronotype is heavily dependent on their genotype in clock genes and, in many populations, polymorphisms in the PER3 circadian rhythm gene show a strong correlation with diurnal preference. In particular, a variable nucleotide tandem repeat (VNTR) whereby individuals either have four or five repeats of a 56 base pair sequence, has been strongly associated with diurnal preference, those with the shorter allele tend to be 'morning people' and those with the longer allele 'evening people' [14,15]. This association is even stronger in those individuals who suffer from sleep-related disorders, such as delayed sleep phase syndrome [14,16]. In addition to the 4/5 VNTR, other polymorphisms in the PER3 gene have also been associated with diurnal preference, including various single nucleotide polymorphisms (SNPs) and a further VNTR located in the promoter region of the gene [17–20], implicating the expression and function of PER3 in the mechanism underlying diurnal preference.

Other studies have also associated further clock genes that could play a role in whether an individual is a morning or an evening person, such as ARNTL2 and NRLD2 [19,21].

Strong associations with 'morningness' and 'eveningness' have also been found when looking at a combination of clock gene polymorphisms [22].

Diurnal preference has been implicated in a variety of health problems from sleep, mood and mental health disorders to behavioural issues and poor performance [8–10,23,24]. Not only this, the circadian cycle and the genes associated with diurnal preference have been implicated with the onset of cancer, also affecting how cancer treatments are delivered [25,26].

Chronochemotherapy

From the late 1980s, various clinical trials sought to test the delivery of a variety of chemotherapeutics while considering an individual's circadian cycle. This aimed at reducing the toxicity of chemotherapy while optimising the dose, i.e. chronomodulation, although the evidence for a circadian effect is of variable quality.

Cisplatin was one of the first drugs for which chronotherapy was investigated, to try to reduce nephrotoxicity in cancer patients without compromising its anticancer activity. After rat studies highlighted the circadian clock as having the potential to improve therapy, William Hrushesky's group found similar results in human participants [27]. This led to subsequent studies by the same group that found that adjuvant chemotherapy combining doxorubicin and cisplatin drugs in a circadian-timed treatment plan could reduce cancer recurrence in patients with bladder cancer [28]. However, this was only a small study consisting of just 13 patients [28]. Around the same time, Francis Lévi and his group [29] also found that, in patients with bladder cancer, the levels of fluorouracil detected in the body after continuous-infusion chemotherapy showed circadian variation, but again they only looked at a small number of patients (seven in total). Therefore, the suggestion that bladder cancer patients could benefit if chemotherapy was delivered by a chronomodulated schedule is unclear due to the small patient numbers and has not been studied subsequently.

Further work by Hrushesky looked at the circadian infusion of floxuridine for widespread cancer and found that drug toxicity was reduced, and a higher dose could be tolerated to make treatment more effective [30]. Whereas Lévi *et al.*'s other clinical studies [31–37] primarily aimed at improving treatments to those living with metastatic colorectal cancer and advanced ovarian cancer based on the activity of the drug, including the time of day that normal cells were in DNA synthesis (S) phase of the cell cycle. The proportion of healthy cells in S phase are lowest during the middle of the resting period (in the early hours of the morning) and so delivery of S phase-specific chemotherapeutic agents, such as fluorouracil, at this time can reduce the toxicity of the drug to normal cells [35,36].

Using this biological reasoning, Lévi's studies have also highlighted the benefits of chronomodulated delivery of the combination of fluorouracil, folinic acid and oxaliplatin to deliver maximum doses at circadian-relevant times rather than a standard constant-rate chemotherapy delivery schedule [34,36]. This work then led to additional trials looking at an intensified regimen of the same drugs, further improving the response rate and survival in colorectal carcinoma patients with metastases [37].

Lévi *et al.* [33,38] have also confirmed the clinical antitumour activity of oxaliplatin on a chronomodulated delivery schedule for colorectal cancer, breast cancer and liver cancer and combining chronomodulated delivery of doxorubicin and oxaliplatin in patients with advanced ovarian cancer has significantly decreased chemotherapy-associated toxicities [31].

Other groups have also investigated the effects of circadian schedules for drug infusion. Findings from various pharmacokinetics studies showed that the levels of drugs such as fluorouracil and doxorubicin detected in patients receiving chemotherapy showed circadian variation [39–42]. Benefits of chronomodulated floxuridine for reduced toxicity and better response in colon cancer, gastric cancer and renal cell carcinoma patients were also found [43–45]. Although it is worth noting that, for renal cell carcinoma, response was much less improved in comparison with other cancers (a partial response rate of just 14%) [45], and its resistance to chemotherapy is now well-documented. Work also gained traction looking into how chronomodulated drugs could be used in combination to further improve the success of treatments for endometrial cancer, breast cancer and adenocarcinoma and for gastrointestinal carcinoma patients [46–49].

Work into chronomodulated chemotherapy is still ongoing; more recent studies have been continuing to investigate the benefits and pharmacokinetics of fluorouracil and cisplatin [50–52].

Chronoradiotherapy

In light of the success with chronomodulated chemotherapy, research is beginning to look at chronomodulated radiotherapy or 'chronoradiotherapy'. Thirteen studies to date have looked at the outcome of radiotherapy treatment at different times of the day. Table 1 summarises the main results from each of the studies, organised by cancer type.

Of the 13 studies, five groups were able to find a significant difference in radiotherapy side-effects (acute and late) for patients living with breast cancer, cervical cancer and prostate cancer [53–56,58]. Six groups found significant differences for local tumour control and overall survival for patients living with breast cancer, prostate cancer, colorectal cancer and bone and brain metastases [54,58,59,61,63,65]. Groups that looked for a time-of-day effect in head and neck cancers failed to identify any significant differences [57,60,62,64].

Of the 13 studies, nine groups were able to find a significant difference in radiotherapy side-effects (acute and late), local tumour control and overall survival for patients living with breast cancer, cervical cancer, prostate cancer, colorectal cancer and bone and brain metastases [53–56,58,59,61,63,65]. Groups that looked for a time-of-

Table 1
Literature investigating chronoradiotherapy. Summary of the currently (as of 10 January 2019) published literature that investigates the effects of chronoradiotherapy on treatment response. Nine studies have found an association between the time of day of radiotherapy response and toxicity in a variety of cancers

Reference	Cancer type	Number of patients in the study	Toxicity	Time of day (h)	Any time-of-day differences?	Significance?
[53]	Breast cancer	878 women (across 2 cohorts)	Acute effects* Late effects†	Before 1200 After 1200	Higher incidence of worse reactions in the morning	Significant ($P = 0.03$)
[54]	Breast cancer	395 women	Acute skin reactions‡ Survival outcomes	Before 1000 After 1500	Higher incidence of ≥grade 2 skin reactions after 1500 h	Significant ($P = 0.0088$)
[55]	Cervical cancer	67 women	Acute toxicity	0900–1100 2100–2300	Higher incidence of severe haematological toxicity at 2100–2300 h	Significant
[56]	Cervical carcinoma	219 women	Gastrointestinal mucositis (diarrhoea)‡	0800–1000 1800–2000	Higher incidence of mucositis at 0800–1000 h Higher incidence of ≥grade 3 mucositis at 0800–1000 h	Significant ($P < 0.01$) Significant ($P < 0.05$)
[57]	Head and neck carcinoma	177 total	Mucositis (ulceration)‡ Treatment response§	0800–1100 1500–1800	Higher incidence of ≥grade 3 mucositis at 1500–1800 h	Not significant
[58]	Prostate adenocarcinoma	409 men	Gastrointestinal and genitourinary acute toxicity* Biochemical failure-free survival	Before 1700 After 1700	Higher incidence of ≥grade 1 reactions after 1700 h Worse biochemical failure-free survival after1700 h	Significant (gastrointestinal reactions, $P = 0.01$; genitourinary reactions, $P < 0.001$; biochemical failure-free survival, $P = 0.05$)
[59]	Rectal cancer	155 total (110 men, 45 women)	Tumour response	Before 1200 After 1200	Higher incidence of complete/moderate pathological response after 1200 h	Significant ($P = 0.035$)
[60]	Squamous cell carcinoma of oral cavity/pharynx/larynx	205 total	Mucositis‡	0800–1000 1600–1800	Higher incidence of ≥grade 3 mucositis at 1600–1800 h	Not significant
[61]	Bone metastases	194 total (121 men, 73 women)	Clinical response‖	0800–1100 1101–1400 1401–1700	Females: improvement in pain at 1101–1400 h	Significant ($P = 0.03$)
[62]	Brain metastases	755 total (357 men, 398 women)	Overall survival	0800–1100 1101–1400 1401–1700	No overall difference observed between treatment times	
[63]	Non-small cell lung cancer brain metastases	437 total	Local control Overall survival	0912–1140 1141–1802	Higher local control and overall survival at 0912–1140 h	Significant ($P = 0.016$ and $P = 0.012$, respectively)
[64]	Non-small cell lung cancer brain metastases	172 total 81 men 91 women	Local control Overall survival	Before 1200 After 1200	No differences observed between treatment times	
[65]	Non-small cell lung cancer brain metastases	97 total	Local control Overall survival	1000–1230 1230–1500	Higher local control and overall survival at 1000–1230 h	Significant ($P = 0.014$ and $P = 0.025$, respectively)

Toxicity grades were based on the following scales: *CTCAE, †LENT-SOMA, ‡RTOG, §WHO criteria, ||RECIST.

day effect in head and neck cancers failed to identify any significant differences [57,60,62,64].

Only one study identified the genetic association between radiotherapy toxicity and the time of day. Breast cancer patients who had received adjuvant radiotherapy were assessed for both acute and late toxicity after receiving most of their radiotherapy fractions before or after 1200 h. In addition to this, patients were genotyped for three clock gene polymorphisms: a VNTR polymorphism in PER3, a SNP in CLOCK (rs1801260) and a SNP in NOC (rs13116075). In addition to finding that patients experienced worse side-effects if they received radiotherapy in the morning, the genetic analysis found that this effect was the strongest when patients had a particular genotype – homozygous for the 4 allele of Per3 and/or homozygous for the A allele in NOC [53].

A further study (not listed in Table 1) also looked at the effects of timing on treatment response after radiotherapy by analysing the expression of circadian rhythm genes in biopsy tissue of rectal cancer patients. Results showed a significantly higher expression of core clock genes such as *Clock*, *Cry2* and *Per2* ($P < 0.05$) and the downstream target gene *c-Myc* ($P < 0.05$) in patients with complete tumour regression [66], highlighting the role that an individual's circadian cycle may have on their treatment outcomes.

However, even taking all of this into consideration, treatment variables such as type of radiotherapy, time of treatment, number of fractions and dose, etc. and patient variables including gender, age, cancer type and grade make it difficult to compare results between studies. The Bjarnason *et al.* [60] study included in Table 1 is a good example of this. Overall, there was a higher incidence of grade 3 (or higher) mucositis in head and neck cancer patients when treated in the afternoon (between 1600 and 1800 h), but this did not reach statistical significance. However, when different subgroups were considered in the analysis, significant differences between the two time groups were found for weight loss and mucositis in patients who were smokers or received the highest radiation doses [60].

To design studies to try to reduce variability and make them more comparable, it is important to further investigate the mechanism underlying the observed time-of-day effect to design clinical trials that would give definitive results.

Mechanisms

There are a variety of potential mechanisms that could explain the time-of-day effect that has been observed in previous research.

Hormones

The circadian cycle is important for the homeostasis of the endocrine system, affecting a multitude of hormone secretions. The most relevant of these secretions to circadian rhythm are melatonin and cortisol.

Melatonin is mainly secreted from the pineal gland in the brain, reaching peak levels in the early hours of the morning [67–69]. Melatonin levels are often used to determine the phase of a person's circadian cycle [67–69]. Its primary function is to regulate the sleep–wake cycle, with higher levels contributing to sleepiness in individuals, but is also involved in regulating the immune system and is an important antioxidant in the body [67–69]. There are, therefore, several ways that melatonin levels might help to protect normal tissues against the damaging effects of radiotherapy, including the detoxification of free radicals [67–69].

In the morning, the stress hormone cortisol is secreted in its highest levels from the adrenal glands, thought to prepare the body for waking [69]. Like melatonin, the levels of cortisol can be used to identify the phase of a person's circadian cycle [69]. Due to its role in a variety of metabolic functions and its involvement in the immune response, both melatonin and cortisol levels could contribute to the time-of-day effect observed for radiotherapy side-effects.

Immune Response

Many studies have shown that the immune system is regulated in a circadian manner, with the levels of circulating immune cells oscillating through a 24 h period. The innate immune response that occurs when the body is exposed to any foreign antigen is highest during the day when there are higher numbers of circulating granulocytes, monocytes and natural killer cells [70–72]. By contrast, the adaptive immune response (which happens after re-exposure to a previous antigen) has been observed to be highest during the night, when there are higher numbers of lymphocytes (T and B) [70–72]. At the molecular level, it has also been found that immune cells show rhythmicity in the expression of clock genes [70,72].

Taking the above into consideration, it is perhaps unsurprising that autoimmune and inflammatory diseases have been associated with aberrant circadian rhythmicity. Peripheral blood mononuclear cell isolation and analysis has found that the expression of important clock genes is altered in patients with inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, as well as those with polyglandular autoimmune syndrome (type III) in comparison with healthy controls [73,74].

After radiotherapy, many side-effects, such as mucositis, diarrhoea, lymphoedema, erythema and fibrosis, are the result of an inflammatory immune response and so could be explained by the circadian variation in the immune system [75].

Cell Cycle (including DNA Damage Response/Repair and Apoptosis)

Another possible mechanism for the time-of-day effect of radiotherapy side-effects could be as a result of circadian variation in the cell cycle, in turn affecting the DNA damage response, repair and apoptosis.

The cell cycle works under the tight regulation of cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors to ensure that each step of the cell cycle is carried out fully, passing checkpoints before progressing into the next phase [25,76]. In many tissues, cell cycle proteins that are involved in cell cycle progression, cell proliferation and apoptosis show circadian rhythmicity [25,76]. As a consequence, cells can be cycling at different stages, expressing different cell cycle proteins depending on the time of day [25,76].

As cells progress through the cell cycle, radiosensitivity changes. In skin, most cells are in the more radioresistant DNA synthesis (S) phase of the cell cycle when there is greater exposure to the sun [77]. Considering that these cells are progressing through S phase and M phase during the day, it is very likely that radiosensitivity varies over the course of 24 h [25,76,78]. For fibroblast and keratinocyte cells, key cell types involved in the development of acute skin reactions after radiotherapy, this has been shown to be the case with higher peaks of circadian activity related to DNA replication and cell division during the later hours of the day [78,79]. Furthermore, late effects such as atrophy and fibrosis are a result of rapid cell loss or proliferation, respectively, and so could be explained by variation in the cell cycle [75].

All in all, the suggested mechanisms all have the potential to explain the observed circadian variation in radiotherapy toxicity and response.

Conclusion

Future research should focus on how side-effects that develop after a course of radiotherapy treatment may be improved by exploiting the time of day that therapy is delivered. In view of the success of chronomodulated chemotherapy and the more recent studies into radiotherapy toxicity, it seems likely that radiotherapy outcomes would also benefit from chronomodulation. As such, 'chronoradiotherapy' could offer a promising new way to improve the outcomes of cancer treatment, cheaply and effectively. To date, only a single study has associated genetic variants with time-of-day differences in radiotherapy outcomes and so further work is needed to elucidate a potential mechanism underlying this effect to predict which patients might benefit the most from chronomodulated radiotherapy.

Furthermore, few studies have looked at whether there are differences in circadian gene expression between tumour cells and normal cells, despite current evidence suggesting that there is a misalignment of the circadian clock between healthy and cancerous tissue [80–82]. If this is the case, then this also highlights the benefits that tailoring therapy according to the time of day might have on cancer treatments.

Both research into tumour circadian clocks and identifying the underlying biological mechanisms for the time-of-day response would allow better-informed decisions on the best times of day to test chronoradiotherapy for implementation into clinical trials.

Conflict of interest

The authors declare no conflict of interest.

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