Reviewer's report

Title: Moderate effect of c-MYC expression on response to radio- and chemotherapy in childhood medulloblastoma

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Reviewer: Karel Zitterbart

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In this article, authors used DAOY and UW228 human MB cells engineered to stably express c-MYC and tested whether c-MYC expression has an effect on radiosensitivity and chemosensitivity, using the MTS assay, clonogenic survival, apoptosis assay, cell cycle analysis and western blot. They also analyzed c-MYC mRNA expression in formalin-fixed paraffin-embedded tumor samples from patients with postoperative residual tumor and compared c-MYC mRNA expression with response to radiotherapy and chemotherapy.

Daoy and UW228 cells expressing high levels of c-MYC were significantly more sensitive to cisplatin and etoposide treatment than DAOY and UW228 cells expressing low levels of c-MYC. However, c-MYC mRNA expression was similar in MB clinical samples of responders and non-responders to chemotherapy or radiotherapy. Thus, authors concluded that c-MYC sensitize MB cells to some anti-cancer treatments, however, they failed to show evidence for such an effect on postoperative residual tumors.

Authors used previously established Daoy and UW228 MB cell lines that stably overexpress c-MYC (Stearns et al., 2006). c-MYC mRNA levels, measured by RT-PCR, were 10- to 30-fold in the DAOY-myc and UW228-myc lines compared with either the parent or the empty vector-transfected lines. These c-MYC overexpressing cell lines had higher rates of growth and apoptosis as well as significantly improved ability to form colonies in soft agar compared with control. Xenograft tumors with high levels of c-MYC in DAOY cell line background were 75% larger than those derived from control. Overexpression of c-MYC was also required for tumor formation by UW228.

Authors wanted to evaluate the results of their previous study (von Bueren et al., 2009) where they demonstrated that siRNA-mediated c-MYC downregulation in D341, D425 and DAOY MB cells inhibits cellular proliferation and clonogenic growth, inhibits G1-S phase cell cycle progression and decreases human telomerase reverse transcriptase (hTERT) expression and telomerase activity. Additionally, they found that downregulation of c-MYC reduces apoptosis and decreases the sensitivity of human MB cells to IR, cisplatin and etoposide. They concluded that in human MB cells, in addition to its roles in growth and proliferation, c-MYC is also a potent inducer of apoptosis. To validate these results, they analyzed the response of DAOY and UW228 cells engineered to stably express different levels of c-MYC to irradiation and to panel of chemotherapeutic drugs in this study.
The results described by von Bueren et al. are in agreement with many previous studies. It is well-known that c-Myc has a central and necessary role in the proliferation of normal cells. Following mitogenic stimulation of quiescent cells, Myc is rapidly induced and remains elevated, suggesting that it is required for continuous cell growth. Induction of Myc is sufficient to drive quiescent cells into the cell cycle (Eilers et al., 1989), while inhibition of Myc can block mitogenic signals and facilitate cell differentiation (Heikkila et al., 1987; Holt et al., 1988; Sklar et al., 1991; Sawyers et al., 1992; Hanson et al., 1994).

In the early 1990s Cleveland and Evan and their colleagues established definitively that c-Myc can also activate apoptosis (Askew et al., 1991; Evan et al., 1992). Toxic effects of elevated c-Myc expression were noted by many investigators in the 1980s and reported by several laboratories (e.g. see studies cited in Packham and Cleveland, 1995). However, before apoptosis became accepted as a bona fide cellular process, the toxicity of deregulated c-Myc was not conceptualized as a potential function. The capacity of c-Myc to drive apoptosis in vitro was first credibly established under growth limiting conditions where its expression was enforced and uncoupled from growth factor controls. Thus, following growth factor withdrawal, cells that contain normal c-Myc downregulate its expression and exit the cell cycle, whereas cells where c-Myc is enforced maintain its expression and undergo apoptosis (Evan et al., 1992).

Nowadays it appears that c-Myc is required for efficient response to a variety of apoptotic stimuli, including transcription and translation inhibitors, hypoxia, glucose deprival, heat shock, chemotoxins, DNA damage, and cancer chemotherapeutics (Evan et al., 1992; Harrington et al., 1994a; Wagner et al., 1994; Yao et al., 1995; Alarcon et al., 1996; Graeber et al., 1996; Jiang et al., 1996; Kang et al., 1996; Li et al., 1996; Dong et al., 1997; Koumenis and Giaccia, 1997; Zhan et al., 1997; Nesbit et al., 1998; Rupnow et al., 1998; Shim et al., 1998). Evan and Littlewood have proposed the idea that c-Myc does not act as a death effector but instead acts to sensitize cells to a variety of apoptotic triggers (Evan and Littlewood, 1998). Its role in death by so many stimuli supports the hypothesis that c-Myc has intrinsic function related to cell death. These facts strongly support a dual function model for c-Myc as a coordinate activator of cell proliferation and apoptosis.

In summary, c-MYC plays a dual role and has the ability to drive both proliferation and apoptosis. In situations of cellular stress such as growth factor deprivation, hypoxia, ionizing radiation, or exposure to chemotherapy, c-MYC deregulation may induce apoptosis. However, in childhood MB, high c-MYC mRNA expression, c-MYC gene amplification, and low-level copy changes of c-MYC have been shown to indicate an unfavorable prognosis (Grotzer et al., 2001; Rutkowski et al., 2007; Scheurlen et al., 1998; Zitterbart et al., 2010).

Thus, in accordance with this work by von Bueren et al. 2010, although c-MYC could act as a potent inducer of apoptosis, it seems that in vivo this mechanism does not work in MB and c-MYC has predominantly proproliferative and oncogenic function. However, there is a question if it might be possible to modulate the pro-proliferative function of c-MYC to pro-apoptotic in vivo.
The study by von Bueren et al. is well-written, suitable methods were properly used to analyze apoptosis and cell cycle.

The results describing relationship between c-MYC and apoptosis in vitro obtained in this study are not unexpected. However, taken together with the previous study of the same authors, this is the first time when the relationship between high c-MYC expression and apoptosis in cell lines was compared with mRNA expression and chemosensitivity/radiosensitivity in MB in vivo.

Major Compulsory Revisions

1) The title is relatively misleading. The phrase “Moderate effect …” does not adequately express observed differences between in vivo and in vitro effect of high MYCC mRNA expression on irradiation or cytostatic treatments.

Minor Essential Revisions

1) Abstract, Results, line 5: I would prefer “similar in primary MB samples of responders …”

2) Abstract, Conclusion, line 1: “c-MYC sensitizes MB cells to some anti-cancer treatment in vitro.

3) Methods, Part MB patients and therapy: It should be clarify that FFPE MB samples were pretreatment (i.e. not second surgery samples)

Discretionary Revisions

1) page 15, line 19: the source of reference human cerebellum RNA should be noted

2) I would recommend using more than only two MB cell lines, and to make also transient transfections to exclude the possibility that observed effects could be caused by deregulation of other genes due to incorporation of plasmid with strong CMV promoter.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interest.