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Using Existing *In Vitro* Structural Alerts For Chromosome Damage To Predict *In Vivo* Activity And Direct Future Testing

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While the *in vivo* genotoxicity of a compound may not always correlate well with its activity in *in vitro* test systems, for certain compound classes a good overlap can exist between the two endpoints when an appropriate *in vivo* test protocol is employed. The difficulty, however, lies in establishing the cases for which this relationship holds true and in determining the most appropriate *in vivo* test protocol for a given class. With this in mind, a project was initiated in collaboration with the National Institute of Health Sciences (NIHS) of Japan in which existing structural alerts for *in vitro* chromosome damage in the expert system Derek Nexus (DX) were assessed for their relevance to *in vivo* activity. Initially, the predictive performance of each *in vitro* alert was assessed against *in vivo* data, taken from the *in vivo* micronucleus and chromosome aberration tests.
Structural alerts with the best correlation between the endpoints and most available data were selected for further analysis. An expert assessment was then made regarding the relevance of the *in vitro* alert *to in vivo* activity, information regarding the findings from specific *in vivo* tests was added to the alert and any correlations between activity and test protocol were noted. In some cases correlations between structure and activity in different *in vivo* assays could also be rationalised in terms of the mechanism leading to genotoxicity. Data sources used in this work included a data set of micronucleus test results collated by the Mammalian Mutagenesis Study Group (MMS) of Japan, along with the Vitic Nexus and Leadscope Enterprise databases. A total of 24 *in vitro* alerts were investigated. The modifications made to these alerts led to a significant improvement in the coverage of *in vivo* chromosome damage in DX, as measured against the MMS data set. In addition, the detailed information relating to the activity of the compounds in different *in vivo* test protocols provided within the alerts as well as the rationalisation of these results may prove useful in directing further testing of compounds which activate these alerts.

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Responses of pluripotent stem cells to DNA damage: p53-dependent growth inhibition and de-undifferentiation

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Cancer stem cells are implicated in tumor heterogeneity, recurrence, and drug resistance. However, the origin of cancer stem cells has not been clarified yet. To elucidate carcinogenic mechanisms of stem cells, we introduced the DNA adduct formation system in mouse embryonic stem cells (mESCs) by treating with 7,12-dimethylbenz[*a*]anthracene (DMBA), followed by determination of cell viability and expression of related marker genes. In addition, the responses of mESCs to DMBA were compared with 3-methylchoranthrene (3-MC), a weak DNA adduct reagent. The DMBA treatment decreased colony size and undifferentiated marker gene expression. 3-MC showed almost no effect on mESCs, therefore DNA adduct formation would be responsible for these responses. Furthermore, pretreatment of tumor suppressor p53 inhibitor prior to DMBA addition abolished the growth inhibition and 'de-undifferentiation' of mESCs by DMBA. These data suggest that p53 plays key roles in maintenance of stem cell integrity.

多能性幹細胞の遺伝子損傷応答：p53 依存的な細胞増殖抑制と脱未分化

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がん組織は多様な細胞集団であり，この多様性を支える背景には「がん幹細胞」の存在が考えられる。このがん幹細胞の形成には「幹細胞のがん化」が指摘されており，がん幹細胞形成機構の解明はがんの予防あるいは治療を発展させるうえで重要である．本研究では，幹細胞が発がん性化学物質に曝された時の防御応答について解析した。発がん性ジメチルベンズ[*a*]アントラセン（DMBA）はマウス胚性幹細胞（mESCs）に対して用量依存的に DNA 付加体形成を誘発し，同時にコロニーの形成不良（細胞増殖不良）を引き起こした。一方，DMBA と同じ母核を持つ 3-メチルコラントレン（3-MC）で処理すると DNA 付加体は検出されず，細胞増殖もほとんど影響を受けなかった．したがって，DMBA による細胞増殖不良は DNA 付加体形成に起因するものと考えられる．この時，未分化マーカー発現量は DMBA 処理により減少し，3-MC ではほとんど影響が見られなかった。また，これらの影響は p53 阻害剤を併用することにより完全に抑制された．以上のことから，mESCs は DNA 損傷に応答して p53 依存的に細胞増殖を停止し，その結果，未分化状態の維持が困難になった（脱未分化）と考えられる。