Atlas of Genetics and Cytogenetics in Oncology and Haematology

An Introduction to Chromosomal Aberrations

John R K Savage

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(MRC Radiation and Genome Stability Unit, Harwell, Didcot, OX11 0RD, UK)

Introduction

Visible changes to chromosome structure and morphology have played a very important part as indicators of genetic damage in both clinical and cancer studies.

Most of the changes encountered in clinical studies are "secondary" or "derived" aberrations. This is true also in cancer studies, except that here, there is an ongoing production of aberrations, so that in some cells, a mixture of primary and secondary changes is present, and a continuously changing karyotype (true chromosomal instability).

To appreciate these observed secondary changes we need to understand the primary changes from which they are derived, and it is the purpose of this article to provide a brief introduction to them.

Observation

Primary aberrations are those seen at the first post-induction division, when all the parts are present and there has been no selection by passage through mitosis, nor any modification by subsequent chromosome duplication (Savage, 1976).

Most commonly, observation is made at metaphase, using "solid-staining" with dyes which give high-contrast chromatin staining and negligible cytoplasmic coloration. For more critical work, the chromosomes are banded in various ways, which allows chromosome identification, detection of some forms invisible with solid-staining, and offers more precise positioning of the lesion interaction points (Savage, 1977).

Recently, resolution and classification of transmissible forms has been considerably improved by the introduction of fluoresence *in situ* hybridisation (FISH) chromosome "painting" (Lucas et al., 1992; Savage and Tucker, 1996; Tucker et al., 1995) .

Classification of Primary changes

For purely pragmatic and diagrammatic purposes, we can regard the chromosomal changes we see down the microscope as being the result of "breaks" followed by "re-joins" of the chromosome thread. However, we must always remember that, in reality, their origin is much more complicated (Savage, 1998; Savage and Harvey, 1994).

Since the chromosome we see and score at metaphase has two (sister-) chromatids, it is convenient (and conventional) to divide all aberrations into two broad types:

Chromosome-type where the breaks and re-joins always affect both sister-chromatids at any one locus. Examples in Figure 1.

Chromatid-type where the breaks and re-joins affect only one of the sister-chromatids at any one locus (Fig 2).

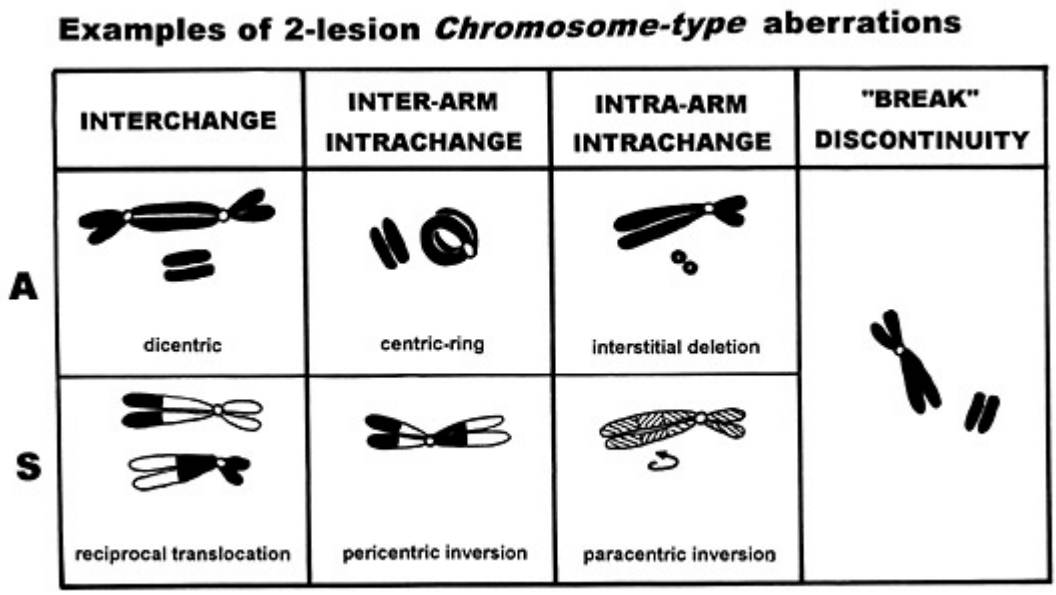

Figure 1

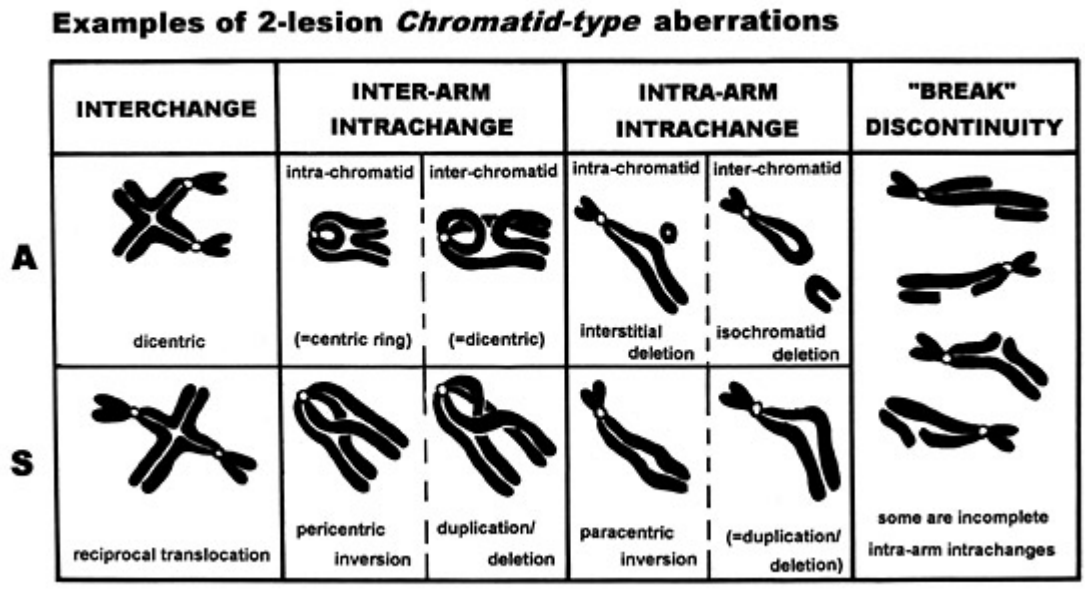

Figure 2

The distinction is important. For some aberration-inducing agents, like ionizing radiation, the type of aberration recovered at metaphase reflects the duplication status of the chromosomes in the treated cell. But, for the majority of chemical agents which can induce aberrations, for ultra-violet light, and most probably all "spontaneous" (and *de novo* aberrations) only primary *chromatid-types* are recovered. When,

at subsequent interphase, the chromatids duplicate, surviving aberrations (and bits of aberrations) are converted into apparent *chromosome-type*s, some of which are then transmitted almost indefinitely to further cell generations. These are the "derived" aberrations, and many are so modified that it is impossible to deduce their primary origin.

Thus, following an "acute" treatment with any clastogen, surviving cells in later generations carry only *chromosome-type* changes. The presence in such cells of *chromatid-type* aberrations is, therefore, an indicator of an ongoing production of primary structural changes, i.e. of some form of chromosome instability.

Nearly all the aberrations we see with solid staining appear to result from the interaction ("re-joining") of two breaks, so we can further classify them on the basis of where these breaks are situated in relation to the chromosome arms (Savage, 1976).

- If the breaks are situated in the arms of different (non-homologous or homologous) chromosomes we have the category of INTERCHANGES.

- If the breaks are in the opposite arms of the same chromosome, we have the category of INTER-ARM INTRACHANGES.

- If the two breaks are both in the same arm of a chromosome, we have the category of INTRA-ARM INTRACHANGES.

These three categories are often referred to collectively as EXCHANGES.

- Finally, some aberrations appear to arise from a single, open break in just one arm. This category we term "BREAKS" or "DISCONTINUITIES". Many (perhaps all) of them are, in reality, intra-arm intrachanges where one end has failed to join up properly, though the limitations of microscopical resolution do not permit us to be certain that the re-joining is really incomplete.

The newer techniques, like FISH chromosome painting, are telling us that a lot of the *chromosome-type* aberrations we see and score as "simple" two-break interactions actually involve more than two breaks, and often more than two chromosomes, i.e. they are COMPLEX EXCHANGES (Savage and Simpson, 1994; Savage and Tucker, 1996; Savage et al., 1984; Simpson et al., 1995). These types have always been known to be fairly frequent for *chromatid-types* , but until the advent of FISH, were always considered to be rare for *chromosome-types.* However, as most complexes will be non-transmissible, and therefore rarely encountered in clinical studies (INSERTIONS, and CYCLICAL EXCHANGES involving several chromosomes, are examples of transmissible complexes), we will not look at them in any detail.

Interaction between the four ends of two breaks can obviously take place in three ways :

- Join back to re-form the original chromosomes ("RESTITUTION") so that no aberration is produced.

- Re-join in such a way that an acentric fragment is always formed (ASYMMETRICAL RE-JOINING, **A**). These forms are invariably visible with solid staining. The fragment (which, if visible microscopically, will contain many megabases of DNA) will be lost at anaphase, and, in the case of INTERCHANGES and INTER-ARM INTRACHANGES, there will also be mechanical separation problems producing "anaphase bridges". Asymmetrical aberrations are therefore almost always cell-lethal, and so rapidly disappear from a population of continuously dividing cells (Lea, 1946; Savage, 1989). Thus, they are rarely encountered in clinical situations where there is not an ongoing induction of aberrations. Instability in cancer cells, however, does lead to the occasional presence of asymmetrical ("unstable") changes.

- Re-join in a way that never leads to an acentric fragment unless one of the re-joins is incomplete

(SYMMETRICAL RE-JOINING, **S**). Many such symmetrical *chromosome-type* exchanges are not visible with solid-staining, and their accurate detection requires special techniques like banding or FISH-painting (Lucas et al., 1992). In contrast, most symmetrical *chromatid-type* exchanges are visible with solid staining because of the retention of sister-chromatid adherence until metaphase. Because there is no loss of genetic material, and no mechanical problems at mitosis, most symmetrical forms are transmissible to future cell generations (Savage, 1976; Savage, 1995), hence they constitute the bulk of the recovered "derived" aberrations encountered in clinical and cancer cytogenetics.

As mentioned, re-joining can sometimes be (apparently) INCOMPLETE. This is much more frequent for *chromatid-type* aberrations (typically 30-50% of interchanges) than it is for *chromosome-type* aberrations (difficult to measure accurately, but probably around 3-5%). Incompleteness leads to genetic loss, and so to increased cell lethality.

The four basic categories discussed above are seen in their simplest forms for *chromosome-type* aberrations, as shown in Figure 1. Traditionally, certain forms have specific names, as indicated. Symmetrical forms are seldom visible with solid staining (probably less than 20% of *reciprocal translocations* lead to an obvious change in chromosome morphology), so, in the diagrams, these forms are shown in two colours, just as they are detected when using FISH chromosome (or arm) painting. There is no reliable method as yet for detecting paracentric inversions.

Because sister-chromatids tend to adhere, strongly, along their lengths, many *chromatid-type* symmetrical forms remain visible without recourse to special staining methods, as Figure 2 shows. Moreover, the presence of sister-chromatids allows additional lesion interactions (*inter-chromatid intra-arm intrachanges*) not possible with *chromosome-type* changes. As a consequence, there is a much higher frequency of *chromatid-type* changes, and a much greater variety of forms, compared with *chromosome-types*, following a given treatment.

Moreover, the interactions within chromosome arms which are now possible make *chromatid-type* aberrations a much more likely source of the complicated duplications/deletions etc. encountered in clinical and cancer studies.

Combinations of the various categories are frequent, especially for *chromatid-type*s (*interchange/intra-arm intrachange* particularly so, giving rise to configurations like "triradials" (Savage and Harvey, 1994)), but these, of course, constitute a type of complex exchange. Surviving remnants of such events are responsible for some of the curious anomalies recorded in clinical and cancer cell studies.

Relationship to the cell cycle

Conventionally, the period between successive mitoses ("INTERPHASE") is sub-divided into three phases G1, S and G2 . For critical work, further sub-division of S is possible (Savage et al., 1984). G1 is the pre-duplication period, when the cell begins to prepare for DNA synthesis and the next mitosis. If the cell is not going to divide again, it passes out of cycle during this phase into another phase termed G0. From this phase it may, or may not, be possible to call it back into a division cycle. Usually, however, cells pass on to irreversible differentiation with their chromosomes unduplicated.

S-phase is a discrete period of interphase of a few hours duration during which the chromosomal DNA and protein is duplicated, and the new chromatin segregated into the sister-chromatids. Each chromosome has a precise programme of replication, closely associated with its G-band pattern.

Pale G-bands always replicate early in S-phase, dark G-bands later, and constitutive heterochromatin tends to be among the very last regions to replicate (Aghamohammadi and Savage, 1990; Savage et al., 1984).

During G2 , the newly replicated chromosomes undergo a rapid programme of condensation, packing and coiling to produce the familiar metaphase chromosomes where we normally identify and score aberrations. These condensed chromosomes facilitate transport of the genetic material to the daughter cells at mitosis. This condensation and packing readily obscures, modifies and disguises aberrations which are produced during interphase - a point that should always be borne in mind when interpreting what we see down the microscope.

Most aberration-inducing agents can introduce lesions into the chromatin at all stages of the cell cycle, but relatively few of them can produce actual structural changes in G1,(and therefore give rise to primary *chromosome-type* changes) or in S and G2 (producing primary *chromatid-types*).

Ionising radiation, restriction endonucleases, and a few chemicals like bleomycin and some antibiotics are amongst those that can.

Almost all remaining aberration producing agents are "S-dependent" ; surviving unrepaired lesions from G1 or G2 have to pass through a scheduled S-phase to convert them into exclusively *chromatid-type* aberrations.

Any interference with or abnormality in the processes of chromatin replication also leads to *chromatid-type* aberrations visible at next mitosis. It is almost certain that the vast majority of "spontaneous" and *de novo* aberrations arise in this way. Chromosome instability syndromes also probably produce aberrations via defective S-phase pathways.

However they are produced, the resulting *chromatid-type* aberrations are qualitatively (but not quantitatively) identical.

Meaningful quantitative work with *chromatid-types* is extremely difficult because observed frequencies fluctuate with time of sample after treatment, and are subject to dramatic modifications as the result of mitotic perturbation and differential cell selection. This makes comparison between different treatments, or the production of sensible dose-response curves, virtually impossible (Savage and Papworth, 1991).

Aberration transmission and stability

Although there is an enormous range of primary aberration forms, very few of them are transmissible to future cell generations long term, so only a handful of secondary (or "derived") forms are recovered (Savage, 1976; Savage, 1995).

The following paragraphs list the kinds most likely to be encountered, together with comments and a note about probable primary origin.

RECIPROCAL TRANSLOCATION :

Involves no mechanical separation problems at anaphase, and usually no genetic loss or imbalance. Problems can occur at meiosis because of multivalent formation, and degrees of sterility may arise.

At the molecular level, the re-joining points can disrupt important genetic sequences, leading to inactivation, mutation or position effects (e.g. the *t*(9;22) Ph1 chromosome of CML).

Derived directly from *chromosome-type* reciprocal translocations or from one segregation sequence of symmetrical *chromatid-type* interchanges. (Note that the alternative interchange segregation leads to imbalance and cell lethality).

PERICENTRIC INVERSION :

Very similar properties to those for reciprocal translocations given above. Large inversions lead to meiotic bridges, sterility and cell death.

Derived directly from *chromosome-type* or *chromatid-type* pericentric inversions.

PARACENTRIC INVERSION :

Very difficult to detect at the chromosome level unless they are very large (many megabases of DNA). Again the re-joining points can disrupt important genetic sequences, and reverse segments of the reading frame. Large inversions will give problems at meiosis.

Derived directly from *chromosome-type* paracentric inversions, or from one form of *chromatid-type* intra-chromatid intra-arm intrachange (Revell-type 3 (Revell, 1959; Savage, 1976; Savage, 1989).

INTERSTITIAL DELETION :

The loss of small segments of a chromosome (usually in only one homologue) is not uncommon. Many mutations that have been genetically sequenced have been shown to be actually small deletions.

Very occasionally, the loss of quite large segments appears to be compatible with cell survival.

Derived directly from *chromosome-type* interstitial deletions ("double minutes") and from the alternative form of *chromatid-type* intra-chromatid intra-arm intrachange (Revell-type 2 (Revell, 1959; Savage, 1976; Savage, 1989)) to that which produces paracentric inversions. Segregation products from some complex *chromatid-type* interchanges can also carry deletions.

TERMINAL DELETION :

It is now questionable whether true stable terminal deletions actually exist. All those that have been investigated using the new fluorescent telomere probes are found to be "capped" by telomere sequences. This either means that they are disguised interstitial deletions, where one re-join point was almost terminal, or that survival has been rendered possible by *de novo* telomere synthesis. The recent development of end-specific telomere probes should be able to solve this question (Boei and Natarajan, 1998; Boei et al., 1998).

Derivation, if genuine, from various forms of incomplete *chromosome-type* or *chromatid-type* intrachanges and interchanges, followed by telomerase activity to achieve capping.

INTERSTITIAL DUPLICATION :

Segments of a chromosome repeated in tandem, sometimes in reverse sequence. This may not necessarily arise from a pre-existing structural aberration ; segment amplification and re-duplication is a well attested phenomenon under certain conditions (e.g. HSR regions following chronic methotrexate exposure). Nevertheless, there are primary aberrations which can survive as segmental duplication.

Most likely derived from one form *chromatid-type* inter-chromatid intra-arm intrachange (Revell-type 1 (Revell, 1959; Savage, 1976; Savage, 1989)). Some forms of complex *chromatid-type* interchanges can segregate to give surviving chromosomes with duplicated segments.

INTERSTITIAL INSERTION :

Deletion of a segment and its insertion into another chromosome within the same cell is a fairly common transmitted aberration. Much less common is the insertion of a segment additional to the two complete homologues within a cell.

All insertions are derived from complex exchanges, since, by definition, their production requires the interaction of a minimum of 3 lesions. Either *chromosome-type* or *chromatid-type* complex interchanges may be involved, the range of inter-intrachanges in the latter being particularly productive of insertions.

Occasionally, a surviving dicentric may be found, usually without the related acentric fragment. Very often, the two centromeres lie very close together, because, under these circumstances, only one of the centromeres is active, so anaphase bridges do not form. Likewise, an occasional centric-ring may survive, again usually very small so that "fall-free" separation always happens. Larger rings are very unstable with respect to size, and the positive selection pressure towards very small rings soon eliminates the big ones.

Most of the above comments apply to the situation in normal individuals and cells. When we turn to cancer-derived cells, or to transformed cell lines growing in culture, the situation is somewhat different. These cells are inherently chromosomally unstable. There is a continuous production of structural change so that new primary changes are superimposed on the already existing background of secondary aberrations, and these new ones, in their turn, become secondary.

Moreover, some of the new changes are being produced in already abnormal chromosomes, so the observed aberrations are often very complicated and bizarre.

On top of this, it is clear that most cancer cells are very tolerant of chromosomal loss, or gain, as is evidenced by considerable numerical variations and multiple chromosome copies. These facts make cancer cytogenetics a very difficult and uncertain field for investigation, and considerable credit goes to those workers whose careful and painstaking efforts have produced meaningful advances.

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Contributor(s)

Written 03-1999 John R K Savage
34 City Road, Tilehurst, Reading, RG31 5HB, UK

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For comments and suggestions or **contributions**, please contact us

jlhuret@AtlasGeneticsOncology.org.